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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

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TITLE OF THE INVENTION (280 characters max)

METHOD FOR IMPROVED TARGETING OF LIGANDS WHEREIN THE LIGAND COMPRISES A TARGETING MOLECULE, ...

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ENCLOSED APPLICATION PARTS (check all that apply)

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METHOD OF PAYMENT

A check or money order in the amount of:

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No
☐ Yes, the name of the Government Agency and the Government Contract Number are:

Respectfully submitted,

J. Philip Polster

Date: July 19, 2002

Reg. No. 24,739

Express Mail No.: EL 820489939 US

EXPRESS MAIL FILING CERTIFICATE

RE: U.S. Patent Application
TITLE: METHOD FOR IMPROVED TARGETING OF LIGANDS WHEREIN THE LIGAND COMPRISES A TARGETING MOLECULE,.....
INVENTOR: Meir Strahilevitz

I hereby certify that this U.S. Patent Application is being deposited with the United States Postal Service utilizing the "Express Mail Post Office to Addressee" service addressed to Commissioner for Patents, Washington, D.C. 20231 on July 19, 2002.

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METHOD FOR IMPROVED TARGETING OF LIGANDS WHEREIN THE LIGAND COMPRISES A TARGETING MOLECULE, TO A SITE IN AN ORGANISM, PARTICULARY A CANCER SITE, COMPRISING AFFINITY BINDING OF AT LEAST ONE OF THE GROUP COMPRISING OF TUMOR CIRCULATING ANTIGEN, ANTIBODY SPECIFIC TO TUMOR ANTIGEN, COMPLEX OF TUMOR CIRCULATING ANTIGEN AND ANTIBODY TO TUMOR ANTIGEN AND ANTIBODIES SPECIFIC TO AT LEAST ONE CONSTITUENT OF THE LIGAND

The field of the invention is targeting of ligands to a site in an organism, particularly a cancer site, by utilizing absorbents with selective or specific affinity to chemical species, wherein the adsorption of at least one molecule is associated with improved targeting to the site in the body, such as a tumor site and increased concentration of the ligand in the targeted site, relative to its concentration in the rest of the body.

The absorbents are primarily incorporated in an extracorporeal device, but the field of the invention is not limited to Extra Corporeal Affinity Adsorption or Extracorporeal Affinity Dialysis (Extracorpreal Affinity Dialysis is a method involving both dialysis (and/ or filtration) and adsorption as detailed in US patent 5,753,227, see in particular; column 5 line 40 to column 7, line 44), in that at least one of the absorbents may be administered to the organism, such as by intravenous intraperitoneal, or other route and by binding of the "adsorbent" to the species, the species is cleared faster then it would otherwise clear from the body of the organism by increased elimination from the body, such as elimination through the kidney or liver or Reticulo Endothelial System (RES), or by increased metabolism and breakdown or neutralization of the chemical species.

The methods and devices that are describing the field of the invention and are relevant to elements of the invention that is the subject matter of the current application, include the following patents, patent applications and publications, all of which, including the references cited in said documents are incorporated herein by reference: US patents 4,375,414 and US 4,813,924 and any and all divisional applications or patents of said patents; US 6,039,946 and US 5,753,227, US 6,264,623 and published US patent application US 2001/0039392A1, US 2002/0019603 Al and all their US divisional

applications and issued patents and all equivalents and foreign counterparts of said patents and the divisional patent applications and divisional patents of said patents; PCT WO96/37516 and its foreign and US counterparts and all divisional applications thereof; and US Provisional applications 60/374715, filed April 23, 2002 (STRM 8363, attached hereto as Appendix A) and 60/381118, filed May 17, 2002 (STRM 8387, attached hereto as Appendix B). For abbreviation all these patents and patent applications will be referred to at times as "my patents". Also incorporated herein by reference are the following patents and publications, including the references cited in them: Nillson et al: US patent US 6251,394 B1, V Pimm: Nucl. Med . Biol. Vol. 22, No 2, pp. 1020-1027, 1993, D. A. Goodwin et al: Antibody Immunoconjugates and Radiopharmaceuticals, Vol. 4, number 4, pp. 427-434, 1991, Van Kroonenburgh et al., Nucl. Med. Commun., Vol. 9, pp. 919-930, 1988, M Gurkavij et al., Cancer Research (Suppl.) Vol. 55, pp. 5874s - 5880s, December 1, 1995. The limitations inherent in methods known to date, for the targeting of ligands, such as Treatment Ligands (TL) and Visualization Ligands (VL) [The definition of these terms is in accordance with PCT application WO 96/37516 its US and foreign counterpart and their divisional applications.], to a site in an organism, particularly, but not exclusively tumor site. As known in the art of targeting ligands to sites in the organism, such sites may be for example, an area of infarct in the heart, or a specific organ such as the Thyroid gland ,as examples. The tumor sites, in accordance with the current invention are both solid tumors that are tumors originating from cells outside the blood and the bone marrow (e.g., Leukemias and Lymphomas) as well as "soft tissue tumors" such as Leukemias and Lymphomas. Examples of solid tumors are well known and include, for example, colon carcinoma ovarian carcinoma pancreatic carcinoma as well as sarcomas., as well as brain tumors, such as Asrocytoma and Glioblastoma .The treated organism which is a mammal, including, but not limited to a human, is conceptualized in accordance with the present invention to contain three compartments: The Tumor Compartment (TC), The Blood Compartment (BC) and the rest of the organism: The Healthy Tissue and Healthy Organs Compartment (HC), including for example, the liver, kidney, spleen and lymph nodes. It should be realized that some or all of the organs may include tumor cell masses (such as metastases of tumor) . As used in the current invention the non tumor tissue and cells of the organ (kidney and liver for example.) will be referred to as HC and the metastatic cells (typically in the form of multiple cells): are referred to as TC, even though they reside in

the kidney or the liver, for example. It is clearly appreciated that the three compartments are in fluid communications, through, but not limited to the interstitial fluid (IF), the capillaries of the blood circulatory system and the fluids of the Nervous System, Cerebro Spinal Fluid (CSF) and peritoneal fluid (PF), for example. Further more, there is also fluid communications between the intracellular fluid (ICF) and the other compartments, including the above fluid compartments, for example the IF. It is also realized, that various chemical and cellular species, such as proteins, peptides, various antigenic and haptenic molecules, whether endogenous to the organism, or administered to the organism (such as TL and VL) various sub populations of lymphoid cells and macrophages, invading microorganisms such as viruses, bacteria and protozoa, are able to move from one compartment to the other, and may be at a steady state balance between the various fluid compartments and TC, BC and HC, meaning that removal of a molecular or cellular species from the BC, may for example change the rate of movement of the removed species from the other compartments to the BC. In accordance with the present invention the tumor cells of the present invention contain Tumor Antigens (TA) and the Blood Compartment (BC) may contain Circulatory Tumor Antigens (CA) . It will be realized in accordance with the discussion above, that the CA, being a chemical species, usually will be able to migrate between the various compartments. A CA may be identical in chemical and antigenic structure to TA or it may have chemical and antigenic structure that is similar to but not identical to TA (for example the CA may have different affinity to specific monoclonal antibodies specific to TA then the affinity of same antibodies to TA). The body of the organism may produce antibodies to the TA and /or the CA. This antibodies are produced by the organism, as the result of the immune system in the organism mounting a humoral immune response directed at the TA and /or the CA . This antibodies are referred to as Native Antibodies (NAB) . NAB may act as "Enhancing Antibodies" (K. A. Hellstrom and I. Hellstrom: In: Encyclopedia of Immunology, I. M. Roitt and P. J. Delves Eds. Academic Press, 1998 p. 2440-2445), such antibodies may bind to TA and mask the TA on the tumor cells, they may bind to lymphoid cell receptors and inhibit their ability to kill tumor cells, or they may participate in inducing suppressor lymphoid cells that inhibit tumor killing by Cytotoxic T cells (CTL) Examples of TA are given in WO 96/37516 and include Carcino Embryonic Antigen (CEA), Le(y), Alpha-Fetoprotein (AFT). Many other tumor antigens which include tumor specific

antigens and developmental antigens (for example ovarian carcinoma CA-125 antigen) as well as monoclonal antibodies specific to these antigens are known in the art. (B. J Van den Eynde and A. M. Scott: in Encyclopedia of Immunology, *supra* pp. 2424-2430 .) In the presence of both CA and NAB, at least some of the CA and NAB bind to each other and produced CA-NAB complex . Such complexes may be in "antigen excess" (CA2NAB), wherein the complex will generally have free antigenic sites available to bind additional antibody molecules, or it may be in "antibody excess" (CANAB2), wherein the complex will generally have free antibody binding sites available to bind additional antigen molecules. CA-NAB, was found to play a role in the etiology and pathogenesis of cancer (F. A. Salinas and M. G Hanna, Jr. Eds. Contemporary Topics in Immunobiology, Vol. 15 :Immune complexes and Human Cancer, Plenum Press, 1985) and removal of CA-NAB by plasma exchange can be therapeutic (Immune complexes and Plasma Exchange in Cancer Patients, B. Serrou and C. Rosenfeld, Elsvier/ North-Holland Biomedical Press, 1981).

Factors that interfere with targeting TAB-bound VL or TL to a tumor site in an organism include:

(1) Presence of CA or CA2-NAB, that competes with the TA for binding of the TAB.

(2) TAB(bound to VL/TL) or CA-TAB (bound to VL/TL) complex binds to receptors such as Fc Receptors. If TAB is toxic, for example, is radioactive or is a toxin or toxic drug, this will lead to increased concentration of toxic VL/TL in healthy organs such as the liver, by binding of the TAB's Fc to Fc receptors on liver cells, when TAB contains Fc, such as when TAB is an intact antibody (it should be realized as mentioned earlier that TAB as defined in the current application may also be an antibody fragment, including synthetic fragment and fragment produced by genetic engineering. Complexes of such fragments with CA, even though they do not have Fc fragment, and do not bind to Fc receptors in the liver, they can still be cleared in the RES in the liver and elsewhere in Reticulo Endothelial System (RES), by RES cells, such as macrophages.

(3) NAB compete with TAB for binding to TA and may inhibit TH1 Helper Cells' immune response to the cancer.

(4) CA-NAB complex concentrates in the RES, liver and kidney, for example, within the HC and toxic effects on normal organs is induced by this concentration.

(5) Particularly, following repeated administration of heterologus targeting antibody (eg: mouse monoclonal targeting antibody to human recipient), the recipient may produce antibodies specific to the targeting antibodies . Such antibodies may be specific to the Fc part of the antibody (antiisotypic antibodies) or they may be directed to the Fv fragment (antiidiotypic antibodies). Such anti-targeting antibody antibodies (ATAA) can be produced by the organism, also when the TAB is a chimeric or humanized monoclonal antibody (M. V. Pimm *supra*, Van Kroonenburgh et al., *supra*.) ATAA may compete with TA for the binding of TAB.

It should be realized that depending on the particular cancer and the individual case, CA, CA-NAB complex, NAB, free ATAA TAB-ATAA complex (after the administration of TAB) may be present in various concentrations in the BC HC and TC. Optimally, particularly when these various concentrations are not known, it may be advantageous to remove or otherwise reduce more then one of the above species, and at least in some situations it may be desirable to remove as many of these species, as possible from the BC and/or HC and/or TC, prior to the administration of the TAB-VL or TAB-TL. Reduction of the amount of one or more of the above species in the BC and/or HC and/or TC, prior to the administration of the TAB-VL or TAB-TL, can preferably be achieved, in accordance with the present invention by Extracorporeal Adsorption, but may include, in some situations, reduction of one or more of the above species, by the administration to the subject of agents that would increase the breakdown and /or clearance from the body compartments of one or more of the above species, that are known to increase such clearance from the organism (see, for example, D. A. Goodwin, *supra*).

One approach that was used to reduce this interference is the administration of unlabeled targeting antibody prior to the administration of the labeled targeting antibody. This approach was associated with limited and various success (see for examples: M Helma et al. Cancer Immunol. Immunother. (1998) 47: 39-46, Beatty, B. G. et al. Cancer Res. 49, 1587-1594, 1989., Ahonen et al.: Acta Oncologica (1993), 32, 7/8, pp. 723-7., Schrijvers A. G. H. et al.: J Cancer Research, (1993) 53, 4383-04390, September 15, 1993. Clearly significant improvement in specificity (e.g.: to target cell, target tissue or organ, relative to the rest of the body) of targeting is needed. Pre administration of unlabeled targeting antibody ,while binding to circulating antigen or to CA2NAB in the blood circulatory system (or in other biological fluid such as for example, cerebrospinal fluid

(CSF) and peritoneal fluid) and thus reducing the amount of subsequently administered labeled antibody in the blood and /or in the liver, by reducing the amount of circulating antigen that can bind the labeled antibody, the pre administration of unlabeled antibody will also lead to the unlabeled antibody binding to the TUMOR ANTIGEN ON TUMOR CELLS and by this mechanism will REDUCE targeting to later administered labeled antibody to the tumor target.

Thus, generally Extracorporeal Adsorption (ECA) is preferred, in view of the fact that such a method is not associated with administration of additional species to the subject (that may have their own toxicity) and is not associated with increased concentration of TAB, TL or VL in healthy organs of the subject, associated with clearance, such as the liver and kidney. Generally the removal of one or more of the above species is completed 15 minutes to 48 hours prior to the administration of the TAB-VL or TAB-TL. It should be realized that the Targeting Ligand may be a species that is not an antibody species and its binding to the targeted site in the organism, may be based on none immunologic binding, as detailed and described in the incorporated references, in particular patens 6,039,946 and 5,753,227 and their counterparts and in Pimm, *supra*, Gurkavich, Supra and Goodwin, *supra*. Thus the targeting ligand may be a ligand that binds none immunologically to a receptor such as Epidermal Growth Factor Receptor, or it may be the peptide hormone Somatostatin, that binds to the Somatostatin Receptor, which is present in high concentration in many tumors. The targeting ligand can also be other targeting proteins or peptides (OTP), or they can be none peptide targeting molecules such as a drug, for example Atenolol that binds to Beta-Adrenergic Receptor and Haloperidol, that binds the Dopamine 2 receptor.

My US patents and patent applications *supra* and in particular PCT applications WO 96/37516 provide for improved targeting over the previous art, by including a step of ECA of a species comprising circulating tumor antigen circulating tumor antigen-antibody complex and circulating (such as enhancing) antibodies specific to tumor antigen.

These methods will not be associated with competition of the administered unlabeled antibodies with binding of the later labeled antibodies to the tumor antigen at tumor sites . This methods will also not include the risks and undesirable effects of administering of unlabeled antibody to the organism, (infection, reaction to foreign protein Immune complex Disease). Optionally the methods of my patents *supra* can also add a

step of removing of the targeted treatment or diagnostic ligand by extracorporeal affinity adorption at a predetermined time following the adsministration of the treatment ligand or visualization ligand to further improve targeting. ECA removal of targeted ligands is disclosed in US patents 6,046,225 and 6,251,394 and PCT application WO 96/37516.

The removal of circulating immune complexes can be achieved in accordance with my patents *supra* by specific extracorporeal immunoadsorbents such as anticomplex antibodies, or by Protein A affinity absorbents (Fresenius Immunosorba ®), Fresenius Prosorba ®, Fresenius C1 q extracorporeal adsorbent Miro ®, Kaneka Selesorb ® as well as Asahi, as disclosed in US patent 4,627,915, as examples. Enhancing tumor antibodies can be removed for exmaple, using affinity absorbents such as tumor antigen, Protein A Immunosorba ®, Prosorba ®, for example, Ciq, (Miro ®, for example) as well as use of the absorbents as used in Selesorb ® and the above Asahi patent. Removal of circulating free tumor antigen (not complexed with antibody) can be achieved by the use of absorbents such as specific antibody to tumor antigen.

It will be realized that tumor targeting inhibitor factors (TIE) may exist in complexes containing more than two molecular species. Such complexes may be affinity labeled for adsorption or adsorbed in the ECA column, by an affinity label or affinity adsorbent specific to any of the components of the complex, or specific to epitopes that are specific to the complex. Examples of such complexes are: CA-TAB-ATAA and NAB-CA-TAB. For example, the complex CA-TABATAA may be adsorbed by antibody to any of its three components, bound non-covalently (e.g., by ligand) to Protein A that is bound covalently to the matrix in the ECA column, or it may be adsorbed by Biotinylated antibody that is bound to Avidin in the Avidin ECA column. Similarly, the CA-TABATAA can be affinity labeled for adsorption, by administering to the subject a Biotinylated antibody, specific to any of its three components, to enable its specific adsorption in the ECA Avidin column or affinity labeled by antibodies to any of the components of the complex (or antibodies to epitopes specific to the complex) and adsorbed by ECA on a Protein A column..

In accordance with the current invention novel devices and methods are provided for the extracorporeal adsorption and removal of molecular and/or cellular Tumor Immunity Suppressor Factors such as: TGFß, p15E and Sialomucin , Suprressor T Cells (K. E. Hellstrom and I Hellstrom Encyclopedia Of Immunology I. M. Roitt and P. J.

Delves Eds. Academic Press 1992, p1530-1531.), Soluble Receptor forTumor Necrosois Factor alpha (s R TNF alpha) and Soluble Receptor for Tumor Necrosis Factor beta (s R TNF beta) , Soluble Receptors for Interleukins 1, 2, and 6 (sRIL-1, sRIL-2, sRIL-6) and Soluble Receptor for gamma Interferon (s R INF-gamma). (M. R. lentz Therapeutic Apheresis Vol 3 (1) p 40-49, 1999 and M. R. Lentz US patent 6,231,536 B1. US patent 6,231,536 provides for Extracorporeal Adsorption of Soluble Cytokin Receptors by affinity lignands bound to matrix, including the optional addition of treatment with untargeted anticancer drugs. The current invention provides for Extracorporeal Adsorption of both Soluble Cytokin Receptors as well as other Molecular Tumor Blocking Factors and Suppressor Cells. In accordance with some elements of the current invention the binding of the affinity adsorbent to the matrix in the Extracorporeal Device is done by binding the adsorbent when the adsorbent is an antibody to the adsorbed (removed) species , to Protein A that is covalently bound to the matrix of the Extracorporeal Device ,through the Fc part of the antibody, or by binding an affinity ligand , such as Avidin , covalently to the matrix and binding the adsorbent covalently to an affinity counterpart ligand (such as binding biotin to the antibody adsorbent or to the cytokin adsorbent). The current invention also provides for the optional use of targeted anticancer treatment ligands including optional use of targeting improvement by extracorporeal adsorption of targeting -inhibitors prior to the administration of the targeted ligand and/or post administration extracorporeal adsorption of the targeted ligand , after its administration.

EXAMPLE 1

STEP ONE OF EXTRACORPOREAL REMOVAL OF CA-NAB, AND/OR NAB AND/OR ATAA PRIOR TO ADMINISTRATION OF TAB-VL OR TAB-TL

Protein A-Sepharose CL-4B obtained from Pharmacia LKB Biotechnology and swollen and washed in accordance with the instruction (Affinity Chromatography . Principles and Methods, Pharmacia Biotechnology Pub., 1991), The Protein A-Sepharose is packed in a column . Preferably the column used is the commercially available Immunosorba ®) sold by Fresenius HemoCare Inc. Redmond, WA. The column requires the use of a plasma separator, as is well known and as recommended by the manufacturer: either a "centrifuge" type, such as Fresenius AS 104 cell separator, Cobe IBM 2997 or membrane type plasma separator, such as Kaneka Sulfox ® or Cobe TPE® can be used to separate, on line, the patient's plasma from the cellular elements of blood. While the

Immunosorba ® column is preferred in some applications, other Protein A Adsorption columns can be used instead. When regeneration is preferred a system including two Immunosorba ® columns with a Fresenius Automatic Regeneration unit, Citem 10 ® are used. When no regeneration of the adsorbent column is needed only a single Immunosorba ® column can be used. When it is preferable to use a disposable column, the column utilized for adsorption can be the Fresenius HemoCare Prosorba ® column in which the Protein A is covalently bound to a silica matrix. It should be realized that other Protein A columns can be used instead, using various biocompatible matrixes to which the Protein A is covalently bound. (Whenever Protein A is mentioned in this application the term includes, Peptide fragments of protein A, including synthetic peptides, that are all known in the art to be used as equivalents of Protein A). The matrixes used include natural polymers, such as cellulose and dextran, various synthetic polymers and copolymers, such as polyacrylamide, polystyrene and polyvinyl polystyrene copolymer, and silica and silicones . One suitable matrix is heparinized silicone described in D. R. Bennett et al. US patent 3,453,194.

The configuration of the matrix is also not limited to a particular form, examples of suitable forms are beads (in particular, spherical in shape), fibrous matrixes ,macroporous matrixes and membranes, including hollow fibers. One possible device is that of a typical multi-hollow fiber filtration, dialysis or diafiltration design. Such a configuration consists of a bundle of hollow fibers encased in a tubular housing. The adsorbent is included in the outer space of the hollow fiber, as disclosed in US patent 5.753,194, see in particular column 8, line 28 to line 68 and figs 2 and 3, the patent uses in the example a chelator as the adsorbent, but in the current invention the adsorbent may be Protein A, either in free form, bound to a matrix, particularly by covalent chemical binding .or alternatively the Protein A can be bound to the membrane, to its blood flow side to the side opposite to the blood flow or to both sides, rather then being incorporated in the outer space of the hollow fiber. The Protein A, in either free form, or matrix bound, can also be physically trapped in the membrane, (such as when the membrane is an unisotropic posysulfone membrane, for example the one produced by Amicon) When the Protein A is encapsulated it can be encapsulated in one of the microcapsules or macrocapsules as described in US patent 5,753,227, in particular column 7 ,line 53-65, or the double encapsulation described by Markus et al. American Heart Journal, Vol. 110 No 1 ,part 1, July 1985, pp. 30-39. In the

practice of the invention adsorbents other then Protein A, or in some situations, in addition to Protein A, can be used, that can adsorb CA, CA-NAB, NAB or ATAA. These adsorbents include for example, TAB (that will bind CA, CA2NAB and ATAA.) Protein G. C1q bound to antiC 1 q antibody, covalently bound to matrix, Clq covalently bound to matrix (for example, Miro ® sold by Fresenius HemoCare) Dextran Sulfate (for example, Selsorb ® sold by Kaneka Corp.) and the adsorbent disclosed in Kuroda et al. US patent 4,627,915. Optionally, the Protein A Extracorporeal column 11 line 7. In the application of this method in accordance with the current invention an intact antibody, or an antibody fragment containing Fc fragment, and specific to a moleuclar species, desired to be removed, in accordance with the current invention, is bound to the protein A in the column by none covalent binding, following procedures known to those skilled in the art (see for example, E. Harlow and D Lane; Antibodies, A Laboratory Mannual, Cold Spring Harbor Laboratory Pub. Pp. 411-522, 1988. and Affinity Chromatography, Principles and Methods, Pharmacia LKB Biotechnology Pub. # 18- 1022-29,, pp. 47-52). In accordance with the present invention the antibodies bound to the protein A in the ECA column are one or more of the following specific antibodies (SA): Antibody to TAB, antibody to CA (for example TAB) antibody to NAB, antibody to ATAA (e. g. anti idiotypic antibody), CA-NAB, NAB (anti idiotypic antibody) antibody to a tumor blocking and tumor immunity suppressor factors and suppressor cells such as: TGF 13, pl5E, Sialomucin, s R TNF alpha, s R TNF beta, s R IL-1, s R IL-2, s R IL-6, s R INF gamma, TH2 T cell epitope. In the EXAMPLE, the organism being treated is a human, the adsorption system used is the Fresenius system containing two Immunosorba ® columns and a Citem 10 ® regeneration unit. Each column contains 62.5 ml of Protein A, which is covalently bound to a cross linked beaded Sepharose matrix. Plasma is first separated from blood cellular elements, using Fresenius AS 104 Cell Separator . The plasma flow rate in the Immunosorba ® column is approximately 20-35 ml/min (flow rates can vary, depending on the individual case and can be in a range from 5 to 50 ml/min, when Immunosorba ®) column is used.

Depending on the individual case and the particular column used, persons with skill in the art can determine without undue experimentation the appropriate flow rate, in the individual case. Step One above can be completed preferably from 0.1 hours to 24 hours prior to the TAB-VL or TAB-TL administration. Most preferably it will be

completed between 0.15 hours to 4 hours, prior to the TAB .administration. The length of step one is typically between 1 hour and 17 hours and more typically between 2 hours and 4 hours, depending on the individual case including, for example, age, weight, presence of pathological fluids in body cavities, such as peritoneum, interstitial space, pericardium and pleural cavity, as examples. It should be understood that while in the EXAMPLE, plasma is being treated, other bodily fluids can be treated, such as peritoneal fluid, lymph, cerebro spinal fluid, with access to these biological fluids achieved by methods that are well known in the art. When fluid of the Blood Circulatory System is accessed and treated, the fluid may be blood rather then plasma (see for example US patent 5,753,227, including use of encapsulated adsorbent, when blood is directly treated) . Following the adsorption STEP ONE, in the EXAMPLE, in STEP 2, the TAB is administered intravenously. The TAB in the EXAMPLE is Hybrid of the two intact monoclonal antibodies Mab CHA-255, specific to the hapten L-SCN-C6H4-CH2-EDTA and Mab ZCE-025 specific to CEA (C. Lollo et al., Nuclear Medicine Communications, Vol. 15, pp. 483-491, 1994) The VL is 111 In-NBEEDTA, which is bound none covalently to antibdy binding site of Mab CHA-255. The hybrid of Mab CHA-255 and Mab ZCE-025 is prepared by using the method described by Lollo et al. *supra*, except that instead of the hybrid F(ab')2, a hybrid of the intact antibodies (which is the preferred species used) is the hybrid utilized. Alternatively it is possible to use hybrid of one intact Mab with the Fab fragment of the second Mab thus including in the hybrid Fc piece of at least one antibody molecules, to enable binding to Protein A and Protein G. used as the adsorbent in the extracorporeal column . US patent 5,753,227, that is incorporated in this application by reference reads in column 10, line 51 to column 11, line 8:

> "When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens. such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of rheumatoid arthritis. for example, the application of the analytical method of J. Goding et al.. J. Immunological Methods. vol. 20, 1978. pp. 241-53, to extracorporeal affinity adsorption in accordance with the present invention. is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the

body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al.. *supra*) is covalently bound to any of the matrixes described in the current invention. for example Sepharose 4B CL The antibody specific to the antigen. for example monoclonal anti-body specific to LDL is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part. and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the ECA column as described in the foregoing examples for the treatment of atherosclerosis, Clearly it is possible to use Protein G instead of Protein A in this system."

The adsorbent used in the CEA column may also be, Mab CHA-255 bound to Protein A or Protein G. through Fc of the Mab, EDTA that is covalently bound to the matrix, preferably through a spacer arm having a length of between 5-20 carbon atoms. Alternatively the EDTA can be conjugated to IgG that will bind to the Protein A through The IgG Fc. The EDTA will adsorb Free 111-In, released in the blood circulatory system. .

A modification of the method is to use liposome incorporation of VL in accordance with WO 96/37516. The incorporated ligand may be TAB-VL (For example, Mab ZCE-025-Mab CHA-255NBE-EDTA-111 In) or it may be any other 111 In containing species, such as EDTA-111 In and target the liposome to the tumor, by binding to the wall of the liposome, covalently, or by ligand (non-covalently) Mab ZCE-025, or its fragment that will target the liposome to the tumor.

EXAMPLE 2

This EXAMPLE is identical to Example ONE above, except that the STEP 3 of ECA of TAB-bound 111 In NBEEDTA111 In, or free 111 In is omitted.

EXAMPLE 3

This EXAMPLE is identical to EXAMPLE 1, except that prior to STEP 1 of ECA, in order to increase the affinity of the adsorbent Protein A, used in the EXAMPLE, monoclonal antibodies specific to the ATAA, (anti idiotypic antiboies to the FV binding site of ATAA), are administered to the treated subject . The production of monoclonal Anti Idiotypic antibodies to ATAA, are well known to those skilled in the art, as ATAA is a complete antigen . while use of monoclonal antibodies, preferably, chimeric or humanized antibodies are preferre, polyclonal antibodies can be used instead. Instead of

administering anti ATAA antibodies, Cold TAB, e.g. TAB that is not bound to a VL or TL, can be administered, instead. In either case the production of ATAA-Anti-ATAA or ATAA-TAB complexes will increase their adsorption by Protein A, hence their effective removal by ECA. It is realized that should TAB be administered it will also inherently have affinity to and bind CA as well as CA2NAB.

EXAMPLE 4

This EXAMPLE is identical to EXAMPLE 1 except that in order to enable the extracorporeal adsorption in STEP 1 of free circulating tumor antigen (CA) that are not bound to NAB and therefor can not be adsorbed by the Protein A used as adsorbent (as well as Protein G or C 1 Q. when these are used as adsorbents) as well as enable the more effective adsorption of ATAA, prior to step 1 of ECA, TAB (e. g. Antibodies specific to CA) is administered (intarvenously, intraperitoneally or by other route, depending on the individual case). TAB will also bind to ATAA (as the Antigen of ATAA) producing ATAA-TAB complex, thus enhancing the adsorption of the ATAA by the Protein A Protein G or C 1 q adsorbent in the ECA column. These TAB antibodies are preferably monoclonal antibodies, preferably chimeric or humanized antibodies, but can be polyclonal antibodies. The amount of antibody administered can be determined by those skilled in the art, depending on the individual case and will generally be between 0.1 Mg/Kg to 2 mg/Kg, Preferably 1 mg/Kg. The time interval between completion of this antibody administration and STEP 1, is relatively short, in order to reduce to minimum the access of unlabeled TAB to the tumor, thus reducing the amount of unlabelled TAB bound to TA, and reducing the competition of unlabelled TAB, with the Labeled TAB . In view of the fact that when unlabeled TAB is administered, such as by intravenous injection, it will have immediate access to CA in the BC, but a relatively delayed access to the tumor site, the time delay between completion of administration of unlabeled TAB and the initiation of STEP 1 ECA, will generally be between 5 minutes and 6 hours, preferably between 10 minutes and 2 hours. The time will depend on the individual case and can be determined by those skilled in the art without undue experimentation.

EXAMPLE 5

This EXAMPLE is similar to EXAMPLES 1-4, except that in order to enable adsorption of free NAB, by Protein A, Anti Idiotypic antibodies specific to NAB are administered prior to STEP 1 of ECA.

EXAMPLE 6

This EXAMPLE is similar to EXAMPLES 1-5, except that in order to enable adsorption of tumor immunity molecular suppressors, (Such as TGF beta , p 15E , Sialomucin, s R TNF alpha, s R TNF beta, s R IL-1, s R IL-2, s R IL-6, s R INF gamma) or tumor immunity cellular suppressors (such as TH2 suppressor cells), antibodies to the suppressor molecules and /or suppressor cells (e. g. In the example , antibodies to Th2 epitopes) is administered, preferably, prior to STEP 1 ECA.

EXAMPLES 7-12

These EXAMPLES are similar to examples 1-6 except that the targeted ligand is a the TL Adriamycin, which is administered in accordance to EXAMPLE 2 of WO 96/37516, incorporated herein by reference.

EXAMPLES 13-19

These EXAMPLES are similar to Examples 7-12 except that the Adriamycin is covalently bound to an intact Mab specific to the Tumor Antigen (TA) alpha-fetoprotein (AFP) and the method is in accordance with EXAMPLE 3 of WO 96/37516, incorporated herein by reference.

EXAMPLE 20 TO 26

The TAB is intact antibody ZCE-025 (see example 1) specific to CEA . It is directly Iodinated with 131 I, using the Chloramine-T (CT) method following the procedure of J. A. Carrasquillo et. al. Cancer Treatment Reports, Vol. 68, No 1, pp. 317-328, January 1984.

The TAB- 131 I is administered in a dose containing 5 to 400 mCi radioactivity. The corresponding amount of Iodinated Mab is 0.65-52 ma. The other parameters of Tab administration are identical to those in EXAMPLES 1 -6 In the above examples the 131 I TAB is used for TREATMENT. The dose used for diagnostics is 5 to 15 mCi . The procedures for adsorption of CA, and/or CA-NAB and/or NAB and/or TAAA and/or TGFß and/or p 15E and/or other tumor suppression including tumor immunity suppression factors are identical to those described in EXAMPLES 1-6.

In all the EXAMPLES 1-26, wherein prior to STEP 1 ECA, the treated organism is administered a species (TAB, Antibody to TAA, Antibody to NAB Antibody to a Tumor Suppression Factor, such as TGFß and p 15 E, for example or any other TSF including TISF) aimed at production of a complex between the administered species and a

Targeting-Inhibitor Species or a Tumor Immunosuppressor species. Rather then administering the species to the treated subject, the species (such as the TAB, antibody to TAA etc. *supra*), which antibodies comprise an Fc piece (preferably the antibodies are intact monoclonal antibodies) The species is bound to the Protein A adsorbent in the ECA column, rather then being administered to the organism, following the general method of using ECA with Protein A or Protein G bound to a specific antibody, in accordance with US patent 5,753,227 incorporated by reference.

EXAMPLES 27 to 52

The procedure for the adsorption of CA and/or CA-NAB and/or NAB and/or TAAA is identical to examples 1-26. The TAB is identical to the TAB of EXAMPLES 20-26, except that the TAB is treated in accordance to the procedures disclosed in US patent 6,251,394, for the labeling of the TAB for post TAB administration adsorption., This patent is incorporated herein in its entirety, by reference. The labeling of TAB is preferably with Biotin.

The TAB may contain any of the Therapeutic or Diagnostic ligands described in the above patent (as well as those described in the current patent application in its entirety, including, but not limited to EXAMPLES 1-26 above and those disclosed in PCT WO 96/37516 .).

The subject being treated for therapeutic or diagnostic purposes is a human subject with a malignant melanoma tumor which is positive for the p97 surface glycoprotein antigen. The TAB is the monoclonal antibody 96.5 specific to p97. The TAB is labeled with 131 I and conjugated to Biotin in accordance with US patent 6,251,394. See column 8, line 66 to column 9, line 19:

> "The monoclonal antibody was the 96.5 (mouse IgG2a) specific for p97, a cell surface glycoprotein with the molecular weight of 97,000 present on 60-80% of human melanoma. The tumor model has been described in detail (Ingvar. C. et al. Nucl. Med 30. 1989, 1224). 2. Conjugation and Labeling of Monoclonal Antibodies. The monoclonal antibody 96.5 (330 ug) was labeled with s 37 MBq iodine-125 (125 b, using the Chloramine-T method. By elusion on a Sephadex G25 column (Pharmacia PD 10) the fraction containing the labeled protein was collected and used for the conjugation. The labeling efficiency of the 125 I 96.5 was around 70%. The radiolabelled monoclonal

antibody was conjugated with biotin by mixing 500 ug of antibody with 41 ug of N-Hydroxysuccinimido-biotin (NHS-biotin) in 0.1 M NaHCO3, 0.15M NaCl with 10% DMSO. The mixture was incubated for 1 h at room temperature, followed by overnight incubation at 4° C. The 125 McAb-biotin conjugate was separated from free biotin-reagent by gel filtration on a Sephadex G25 column, equilibrated with PBS (10 mM Sodium phosphate, 0.15 M NaCl, pH 7.3). The conjugate was stored at 4° C until used.

The dose administered to the subject is the same as in examples 20-26 (5-400 mCi, 0.65-52 mg TAB). 4 to 48 hours, preferably 12 to 24 hours after injection of the TAB, the subject is treated by passing his blood through an Avidin adsorption column, preferably Mitradep ® column produced by Mitra Products, Inc.

The length of adsorption is in most cases between 1 hour to and 10 hours, most preferably, between 2 hours and 4 hours and depends on the individual case, including body weight of the subject and dose of TAB, and can be determined by a person skilled in the art with out undue experimentation. The volume of plasma treated is between 1 and 6 plasma volumes, most preferably between 2 and 4 plasma volumes. The flow rate is between 10-50 ml/min. A scintillation camera is used for imaging, when imaging is desired."

In the step of removal of CA, CA-NAB, ATAA, NAB, TGFß, P 15E , Sialomucin and other molecular and cellular Tumor Suppression Factors (TSF) (included in TSF are Tumor immunity Suppressor Factors (TISF), such as: s R TNF alpha and beta, s R IL 1, 2, and 6 and s R INF gamma, when ever TSF is mentioned , TISF are also included unless specifically excluded , rather then using Protein A as adsorbent, Protein A bound to a specific antibody to CA , CA-NAB, ATAA, NAB and TSF (TSF molecules and/or cells TH2 suppressor cells epitopes, for example), in accordance with the general ECA method, wherein the adsorbent is Protein A bound to a specific antibody, as disclosed in US patent 5,753,227, incorporated by reference. Protein A bound to specific antibody, can be used instead of, or in addition to free Protein A adsorbent (e. g. Protein A unbound to specific antibodies.) Alternatively to the above option, in the step of removal of CA, CA-NAB, ATAA, NAB and other molecular and cellular TSF including TISF , a FIRST affinity adsorbent, other then Protein A or Protein G. can be used for binding to the FIRST affinity adsorbent a SECOND affinity adsorbent, specific to the TSF, in accordance with the

general principle of US patent 5,753,227, in particular column 10, line 51 to column 11, line 80:

"When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens. such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of Rheumatoid arthritis. for example. the application of the analytical method of J. Goding et al.. J. Immunological Methods. Vol. 20, 1978. pp. 241-53. to extracorporeal affinity adsorption in accordance with the present invention. is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al.. *supra*) is covalently bound to any of the matrixes described in the current invention. for example Sepharose 4BCL. The antibody specific to the antigen for example monoclonal anti-body specific to LDL is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part, and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the extracorporeal immunoadsosption (affinity adsorption) treatment column as described in the foregoing examples, for the treatment of Atherosclerosis. Clearly it is possible to use Protein G Instead of Protein A in this system."

In accordance with the general principle of the above method a FIRST adsorbing ligand is covalently bound to the matrix in the column and a SECOND affinity adsorbent, specific to the chemical or molecular species to be removed by adsorption is bound to the FIRST adsorbent by none-covalent chemical binding (chemical binding by ligand) The FIRST adsorbent can be Protein A, for example and the SECOND adsorbent can then be an antibody specific to species to be removed.

Alternatively, for example, the FIRST adsorbent in the ECA column is Avidin or Strepavidin to which is bound a SECOND specific adsorbent, comprising Biotinylated antibody to the species to be removed (STBR) (such as , for example, CA, TAB, ATAA, CA-NAB, NAB, TIF (as previously indicated TSF includes TISF) .

When the TISF is a soluble receptor (such as, for example, , s R TNF alpha , s R TNF beta, s R IL (1, 2, or 6) and s R INF gamma) the adsorbet for the soluble receptor TISF can be either a Biotinylated antibody to the soluble receptor or it can be the biotinylated respective cytokine (TNF alpha, TNF beta, IL 1, 2, and 6 and INTF gamma.) The biotinylated cytokin may be directly bound to the Avidin that is covalently bound to the matrix in the column, or alternatively the cytokin may be fitst covalently bound to a biotinylated carrier molecule such as a protein , a peptide, a polyssacharide moleclule or a polyamine or another polymer molecule that is bound to the Avidin in the column . multiple different SECOND adsorbents , each specific to at least one STBR can be bound to the FIRST adsorbent . Alternatively , some of the STBRs , for example , CA, NAB, CA-NAB, ATAA,TAB can be removed by using only one adsorbent such as Protein A or Protein G, or a specific antibody to the STBR covalently bound to the matrix in the column . Other STBRs can be removed by the use of a FIRST and a SECOND adsorbent as described *supra*, for example , a FIRST adsorbent is Protein A and a SECOND adsorbent is an antibody specific to the STBR, or a FIRST adsorbent is Avidin and a SECOND adsorbent is a biotinylated antibody specific to the STBR.

The use of Avidin-Biotin combination, wherein the FIRST adsorbent is Avidin, was proposed by J Tennval et al. Cancer Suppl. Vol. 80, number 12, pp.2411 -2418,Decemberl 5,1997, for the removal of ATAA, by adsorbing to the Avidin in the ECA column Biotinylated TAB. In accordance with the current invention, this approach can be utilized to remove any molecular or cellular TSF, by binding to the Avidin in the ECA column one or more Biotinylated antibodies specific to one or more molecular or cellular TSF. It should be realized that the general method of US patent 5,753,227, utilizing a SECOND specific adsorbent, bound none covalently (e.g. bound by ligand) to a FIRST adsorbent that is covalently bound to a matrix, is not limited to Protein A-specific antibody or to Avidin-Biotinylated specific antibody pairs. Various pairs of affinity ligands can be used as the FIRST adsorbent and the SECOND adsorbent. The First adsorbent, for example, is an antigen or an hapten covalently bound to a matrix. For example, Dinitrophenyl (DNP) bound to a matrix, preferably through a spacer, or a carrier molecule, such as albumin (e.g. albumin-DNP conjugate bound to the matrix) . The SECOND adsorbent can then be a hybrid antibody specific to both DNP and the TSF. The first adsorbent card be an antibody to DNP and the SECOND adsorbent an antibody to TSF

covalently bound to DNP. The FIRST adsorbent can be an enzyme bound covalently to the matrix in the ECA and the SECOND specific adsorbent will then be an enzyme substrate or enzyme inhibitor conjugated to an antibody specific to TSF (or to another molecular or cellular species targeted for removal.) Similarly any of other known affinity pairs such as, for example, those listed in US patent 6,251,394, column 7, lines 54 to 67, can be used. Such Specific Adsorption methods using a FIRST and SECOND adsorbents can be used not only in ECA treatment, but also in adsorption-based purification or diagnostic methods, to remove any molecular or cellular species from a fluid, including but not limited to biological fluids. as previously noted: whenever "TSF" is mentioned it includes "TISF".

EXAMPLES 53-56

These EXAMPLES are identical to EXAMPLES 5-8 except that the TL is the anti cancer drug Calicheamicin. The subject treated is a human being having a Acute Myeloid Leukemia positive for the CD33 antigen. The TAB is Recombinant Engineered Human Anti-CD33. The TAB is conjugated to the Calicheamicin in accordance to L M. Hinman et al., Cancer Research, Vol 53, pp. 336-3342, July 15, 1993. 3336-3342, The dose of administered TAB-Calciheamicin conjugate is 6-9 mg protein/ m2 (E L Sievers et al., Blood Vol. 93 (11), June 1 1999).

EXAMPLES 50-60

These EXAMPLES are identical to EXAMPLES 1-8, except that the Targeting molecule, in these examples is a none immunologic OTP, the hormone peptide Somatostatin. The treated subject is a human being having a cancer with high concentration of Somatostatin Receptors, as determined by biopsy . (C. Casini Raggi et al. Clin. Cancer Res. Vol. 8 (2), pp. 419-427, Feb 8, 2002.) Adraimycin is conjugated to the Somatostatin in accordance with A. Nagy et al. Proc. Natl. Acad. Sci USA, Vol 95, pp. 1794-1799, 1998, The dose of the conjugate is calculated to contain 30mg to 75mg Adriamycin/ m2 body surface. The post administration of Somatostatin-Adriamycin conjugate clearence from BC and HC is done with an ECA column containing antibody to Somatostatin bound to Protein A. Alternatively, the Somatostatin is conjugated also to Biotin in accordance with C M Eppler et al. J. biol Chem, Vol 267 (22), pp. 15603-12, August 5, 1992 and the conjugate administered to the subject is Biotin-Somatostatin-Adriamycin and the ECA is done with an Avidin column, for

example, Mitra ® ECA column. The subject is treated after post conjugate administration so as to treat 1-5 plasma volumes, ECA is started 1-48 hours after Biotin-Somatostatin-Adriamycin is administered, preferably 2-24 hours . The length of post conjugate administration is 1 -4 hours. The flow through the column is 20-50 ml/min.

EXAMPLES 61-64

These EXAMPLES are identical to EXAMPLES 56-60, except that the subject being treated is administered a Yttrium90 labeled Somatostatin analog prepared according to A Otte et al. The lances, Vol 351, pp. 416-417, February 7, 1998. The dose of the conjugate is 25 mCi to 200 mCi.

EXAMPLE 65

A conjugate of Avidin with Mab ZCE-025 ,which is specific to CEA antigen is prepared according to H. P. Kalofonos et. al.: The Journal of Nuclear Medicine, Vol. 31, No 11, pp. 1791-1796, 1990., except that the Mab ZCE-025 is substituted for Mab HMFG1. The subject being treated is having a tumor positive to the CEA antigen and has circulating CEA antigen in his blood. As determined by standard methods known in the art and available commercially . In STEP 1: The subject is administered intravenously (1 -4 min) 0.1 mg protein/Kg to 2 mg protein/Kg of the Mab-Avidin conjugate. In STEP 2: The subject is treated with ECA with a Biotin Adsorbent Column (BAC) . The BAC ECA starts 0.5h to 48 hours, preferably 2-24 hours after the administration of the Mab-Avidin conjugate. 1 -4 volumes of plasma are treated . The length of the ECA is 1-4 hours . The flow rate is 20 ml/min to 50 ml/min. For its use as the adsorbent in the ECA Biotin is covalently bound to a macromolecular carrier, such as albumin, using the method described in US patent 6,251,394, except that the albumin is substituted for the antibody. Albumin labeling with Biotin can also be done, following the method described in Harlow and Lane *supra*, pp. 340-341, substituting the HAS for antibody. The Albumin-Biotin is covalently bound to Cyanogen Bromide Sepharose 4B beads available from Pharmacia. As an alternative to binding of Albumin-Biotin to Cyanogen Bromide activated Sepharose, The binding of the Biotinylated Albumin to Sepharose can be done by using Avidin-Biotin binding The albumin is biotinylated, so as to have sufficient Biotin moieties conjugated to the Albumin ,thus leaving sufficient number of Biotin molecules in the ECA column to be available to bind to Avidin in the Mab-Avidin conjugate. In STEP 3: The subject is given intravenously 0.5-10 mg protein of the conjugate Biotin -Human Serum Albumin

(HSA)-131 I in 1-5 ml volume of 8.4% Sodium bicarbonate. Human Serum Albumin is directly Iodinated with 131 I according to E. Harlow and D. Lane: Antibodies A Laboratory Manual, Cold Spring harbor Laboratory pub., pp.324-329, except that Human Serum Albumin is substituted for the antibody for the direct Iodination. The preferred Iodination is by the Chloramine T method (E Harlow and D Lane *supra*, 328-329.)

In STEP 4: 0.5-48 hours, preferably 2 hours to 24 hours following the administration of the BiotinHSA-131 I- conjugate, the subject is treated by ECA column ,that includes Avidin as the adsorbent, Preferably the Avidin CEA column is the Mitra ®) ECA column. Length of ECA is 1-4 hours, Flow rate in the ECA is 20-50 ml/min.

The purpose of Steps 1 and 2 is to remove CA, CA2-NAB and TAA from the BC and HC and from the Interstitial Fluid of TC, by specifically labeling CA CA2-NAB and TAA, with the TAB-Avidin conjugate and adsorbing the complexes TAB-Avidin-CA TAB-Avidin-CA2-NAB and TAB-AvidinTAA by adsorption to the Biotin adsorbent in the Biotin ECA column. The Mab in the TAB-Avidin conjugate can be an intact antibody, antibody fragment, including synthetic fragment, and fragment produced by genetic engineering techniques. In addition to the removal of CA, CA2NAB and TAA, removal of any other molecular and/or cellular species can be accomplished by the labeling of the species with a conjugate of Avidin that is conjugated to an antibody to the species, such suppressor species include: NAB, CA-NAB2, Transforming Growth Factor beta (TGFß) pl5 E factor, Interleukin 10 (IL-10), Prostglandin E2 (PGE2), Mucin, Suppressive E Receptor (SER), Immunosuppressive acidic protein (IAP) and adhesion molecules. (K E Hellstrom and I Hellstrom, Encyclopedia of Immunology *supra* and C Botti et al. Int. J Biol. Markers, Vol. 13 (2), pp. 51 -69, 1998.) s R TNF alpha, s R TNF beta, s R IL-1, s R IL-2, s R IL-6, s R INTF gamma (M. R. Lentz ; US patent 6,231,536).

The use of a labeled affinity targeting molecule can be utilized by administration to the subject being treated to affinity label any molecular or cellular species, in particular in the BC but also in the HC and the Interstitial fluid component of the TC, provided that the species targeted for affinity labeling is in equilibrium between the BC, HC and TC compartment (unless the removal is desired only from the BC or from the other treated biological fluid compartment, such as peritoneal fluid CSF or lymphatic fluid, when this fluid is treated in the ECA device, when equilibrium with HC and TC is not required. It should be realized that usually, these species will be in a concentration equilibrium

between the various compartments). With respect to the species that it is desired to remove in the treatment (or diagnosis) of cancer,, in addition to what was listed above: Cellular species include TH2 suppressor T cells that suppress the immune destruction of tumor cells. It will be realized that the disclosed "labeling -based adsorption", will have applications other then in the treatment of cancer, to remove any endogenous or exogenously administered or invading cellular or molecular species, such as auto antibodies in the treatment of autoimmune disease, sepsis associated factors, such as Tumor Necrosis Factor, Leukotrienes, Bradykinin and Interleukin 2, in the treatment of sepsis. Viruses and bacteria as well as protozoa in the treatment of infectious diseases. Toxins: e.g. Tetanus toxin, Butullinum toxin, for example. Other utilizations include: Affinity labeling for ECA of specific cellular and/or molecular species that are collected or harvested for therapeutic use, in the same subject, from which they are removed, or in another subject: For example, removal of specific T cell population, for in vitro treatment followed by readministration to the subject, in the treatment of cancer, e.g. In vitro stimulation of harvested T cells by treatment with Lymphokines ,in LAK or TIL cell treatment of cancer (See for example J. J. Sussman et al., Ann Surg oncol, Vol 1 (4), pp. 296-306, 1994 and S A Rosenberg et al., Science, Vol. 233(4770), pp. 1318-21, Sept 1986.) The only requirement for such affinity adsorption harvesting is the availability or the ability to produce specific affinity labeling reagent that is specific to the target molecular or cellular species, and the ability to conjugate the targeting species to an affinity marker, such as Avidin (or Biotin), for example. (It would be obvious that the method can be modified, by for example, the use of Biotin for Affinity labeling and use of Avidin as adsorbent in the ECA, or the use of affinity pairs other then Avidin Biotin, for example: anti hapten antibody - hapten, Enzyme -substrate and the likes. One significant advantage of the proposed affinity labeling - affinity adsorption (ECA), is that it can be possible to use one single ECA device (Avidin-ECA, Biotin-ECA, for example) to remove many different species form the subject, by using different specific affinity labels targeted to the species to be removed and adsorbing them on the single device used for the ECA step of the method. Alternatively, the different species can be removed at different times or at the same time .

For example: If the species is TAA it can be targeted by TAB-Avidin or by Anti-idiotypic antibody to TAA and remove by a Biotin-ECA. If, the species is Oxidized

LDL it can be affinity labeled by administering a conjugate of Avidin-Antibody to oxidized LDL and removed by ECA with Biotin as the adsorbent. Pairs of affinity labels and their counterparts are known in the art and are disclosed, for example, in US patent 6,251,394, column 6 line 7 to column 8 line 53. Many specific ligands in the Avidin-specific ligand conjugate are known and many are available from commercial sources. Generally, such specific ligands are known by, or can be produced by persons with ordinary skill in the art, following established methods and without undue experimentation. For example, if the specific targeting ligand is a Mab, many of the molecular and cellular species, that are affinity targeted for removal have known Mabs that are specific to them and many of them are available commercially. For example Mabs to CEA and other tumor antigens mentioned in the current application. Alternatively, such Mabs can be produced, without undue experimentation by those skilled in the art, by use of Hybridoma Mab production techniques. As an alternative to administering a affinity labels to the subject, the TSF, including TISF affinity labels can be incorporated in the ECA column, utilizing an ECA method that incorporates as absorbents a FIRST and SECOND absorbents. For example the FIRST adsorbent may be Avidin or Protein A covalently bound to the matrix in the ECA column and the "SECOND" adsorbent may be a plurality of biotinylated antibodies (when Avidin is the FIRST adsorbent, or untreated antibodies (when Protein A is the FIRST adsorbent) that are specific to at least two TSF species. Use of Double Stage Labeling of a tumor for Radiolabeling a cancer was reported in H. P. Kalofonos et al., The Journal of Nuclear Medicine, Vol. 31, no 11, pp. 1791 -1796, Nov. 1990. The authors labeled a lung cancer with a conjugate of a Mab specific to the cancer antigen, that was conjugated to Strepavidin and administered Biotin- 111 In to radio-label the tumor for diagnostic imaging. The authors did not disclose or hinted to the possible use of such labeling for the ECA of molecular and cellular species . Matrixes other then Sepharose can be used to produce matrix-biotin conjugate for use in ECA. For example G. Paganelli et. al. Disclose the production of biotinylated Nitrocellulose and biotinylated Polystyrene (G. Paganelli et al. Int J. Cancer Suool 2, pp. 121-125, 1988.).

Avidin ECA column can be used with whole blood rather then plasma, thus simplifying the adsorption as disclosed by J. Tennvall et al. Cancer Vol 80, No 12 (suppl.) pp. 2411-2418 Dec 15, 1997.

Following ECA on the Biotin adsorbent Column, the subject is administered a VL or TL conjugated to Biotin (it should be realized that the use of Avidin and Biotin in the example can be reversed; with the sequence of : Step l: Administration of Biotin-TAB, Step 2: ECA on Avidin column Step 3: Administration of TL-Avidin or VL-Avidin conjugate. Step 4 ECA on a Biotin column. It would also be realized that affinity pairs other then Avidin biotin can be used instead of Avidin- Biotin pair such as, for example: Hapten -Anti hapten antibody, Enzyme-substrate, Enzyme-Enzyme inhibitor.) The VL-Biotin conjugate can be Biotin- 1 1 1 In and is prepared and administered to the subject according to Kalofonos et al. *supra*: Biotin covalently conjugated to Diethylenetriaminepentaacetic acid (DPTA) is obtained from Sigma chemical comp. St. Louis, Mo and chelated to 1 1 1 In as described in Kalafonos et al. *supra*.

EXAMPLE 66

In STEP 1, the subject is a human with CEA positive cancer, as described in Example 65 is administered a conjugate of Avidin with Mab ZCE-025 specific to CEA.

In STEP 2, The subject is treated with ECA, incorporating biotin as the adsorbent. The purpose of this step is to remove CA, C2 -NAB and TAA.

In STEP 3, An anti-cancer drug, including ,but not limited to one or more of the anticancer drugs disclosed in PCT WO 96/37516 as well as the anticancer drugs disclosed in US patent 5,527,528, incorporated herein by reference is incorporated in a liposome prepared as described in US patent 5,527,528, in particular, example 3 , for the preparation of Avidin coated liposome omiting the last step of incubating the Avidin coated liposomes with biotinilated antibodies. Optionally the Avidin is bound to the Biotin on the liposome wall through a spacer. Suitable spacers are disclosed, for example, in K Hashimoto et al. Biochim Biophys Acta, Vol. 856 (3), pp. 556-65, April 25, 1986. The anticancer drug incorporated into the liposome in the Example is Adriamycin. Step 4, The subject is treated by ECA with Biotin incorporated as the adsorbent, to remove liposomes from the BC, that did not reach or attached to the cancer.

Optionally Step 2, and/or step 3 can be omitted.

Alternatively the liposomes are Biotinilated liposomes of US patent 5,527,528, optionally with the Biotin connected to the liposome wall with a spacer arm, the Adsorbent in the ECA of Step 2 is Aivdin and the adsorbent in the ECA in step 4 is Biotin.

EXAMPLE 67

The subject is the same as in example 66. STEP 1 he is administered Mab ZCE-025, specific to CEA conjugated to Biotin following the method of US patent 5,527,528. In STEP 2, The subject is treated with ECA incorporating Avidin as the adsorbent. In Step 3, the subject is administered Ricin A conjugated to Avidin (L. K. Mahal et al. Science, Vol 276, pp. 1125-1128, 16 May, 1997.)

In STEP 4. The subject is treated with ECA, incorporating Biotin as the adsorbent.

In all of the Examples wherein the administered species is Avidin conjugate of TAB, or the administered species is Avidin conjugate of a none immunologic targeting molecule, in order to remove from the treated subject, when desired any of the species that would inhibit targeting and/or species that would in general suppress the immune destruction, or none immune mechanism destruction of the tumor, such targeting inhibitors and/or tumor destruction inhibitors (TIF , including TISF) can be removed from the BC, HC and TC by incorporating in the ECA adsorption column one or more adsorbents that have specific affinity to the targeting inhibitor or tumor destruction suppressivemolecular and cellular species. This can be accomplished by incorporating such specific adsorbents in a single column, or in different columns, connected in parallel or in series, as disclosed in US patent 5,753,227. When the ECA incorporates Avidin as the adsorbent, The specific adsorbent added to the ECA Avidin column is a Biotin conjugate of a specific affinity ligand (such as Biotin-Mab specific to CA, and /or ATT and/or any of the other suppressors as disclosed in Hellsrom and Hellstrom, And in botti et al. *supra*.) When the adsorbent in the ECA column is Biotin, the Specific adsorbent added is Avidin-Mab specific to the CA and/or ATT, or other target inhibitors or tumor destruction inhibitor (TSF including TISF) cellular or molecular species. as above.

EXAMPLE 68

In any of the Examples 1-67, in the step of removal of specific species from the blood circulatory system or from other biological fluid, such as, peritoneal and CSF, the specific Protein A -Specific Intact antibody adsorbent ligand,can be used for the removal of any substance from a biological fluid source, and for the application of the current invention, can be used for the adsorption- removal of any chemical species that may interfere with targeting, such as CA, CA-NAB, NAB, ATAA, or any molecular or cellular species that suppresses the Immune Destruction, or None Immune destruction of the tumor TSF , including TISF The specific adsorbent is based on the method disclosed in US

patent 5,753,227. Said patent is incorporated in the current application in its entirety. In accordance with the current example, intact antibodies, or antibody fragments, containing Fc,, wherein said antibodies or Fc containing fragments are specific to an epitope on the molecular or cellular species that is to be removed from the biological fluid and BC, HC and TC are added to a Protein A extracorporeal column, such as Immunosorba ® or Prosorba ®, or any other Extracorporeal column that contains Protein A, bound to matrix or encapsulated, as disclosed for example in US patent 5,753,227, *supra*. Such antibodies or Fc containing fragments, in the current example are selected from antibodies, or fragments specific to the molecular and cellular species, that inhibit targeting or suppress tumor destruction as disclosed in the current application and in Hellstrom and hellstrom and Botti et al., and M R Lentz ; Therapeutic Apheresis Vol. 3 (1) p 40-49, 1999.

As disclosed in US patent 5,753,227 and also detailed in Harlow and Lane *supra* ,pp. 519-523, the antibody or fragment will bind to the protein A in the ECA through the Fc of the antibody or fragment, thus producing a specific adsorbent ligand to specifically adsorb one or more of the Targeting-inhibiting molecular species, or molecular or cellular species that inhibit or suppress tumor destruction. This can be accomplished by incorporating such specific absorbents in a single ECA column, or in different columns, connected in parallel, or in series, as disclosed in US patent 5,753,227.

APPENDIX A

METHOD FOR IMPROVED TARGETING OF LIGANDS WHEREIN THE LIGAND COMPRISES A TARGETING MOLECULE, TO A SITE IN AN ORGANISM, PARTICULARY A CANCER SITE, COMPRISING AFFINITY BINDING OF AT LEAST ONE OF THE GROUP COMPRISING OF TUMOR CIRCULATING ANTIGEN, ANTIBODY SPECIFIC TO TUMOR ANTIGEN, COMPLEX OF TUMOR CIRCULATING ANTIGEN AND ANTIBODY TO TUMOR ANTIGEN AND ANTIBODIES SPECIFIC TO AT LEAST ONE CONSTITUENT OF THE LIGAND

The field of the invention is targeting of ligands to a site in an organism, particularly a cancer site, by utilizing adsorbents with selective or specific affinity to chemical species ; wherein the adsorption of at least one molecule is associated with improved targeting to the site in the body , such as a tumor site and increased concentration of the ligand in the targeted site , relative to its concentration in the rest of the body.

The adsorbents are primarily incorporated in an extracorporeal device , but the field of the invention is not limited to Extra Corporeal Affinity Adsorption or Extracorporeal Affinity Dialysis (Extracorpreal Affinity Dialysis is a method involving both dialysis (and/ or filtration) and adsorption as detailed in US patent 5,753,227 , see in particular ; column 5 line 40 to column 7 , line 44) , in that at least one of the adsorbents may be administered to the organism , such as by intravenous intraperitoneal, or other route and by binding of the "adsorbent" to the species , the species is cleared faster then it would otherwise clear from the body of the organism by increased elimination from the body, such as elimination through the kidney or liver or Reticulo Endothelial System (RES), or by increased metabolism and breakdown or neutralization of the chemical species.

The methods and devices that are describing the field of the invention and are relevant to elements of the invention that is the subject matter of the current application, include the following patents , patent applications and publications , all of which , including the references cited in said documents are incorporated herein by reference: US patents 4,375,414 and US 4,813,924 and any and all divisional applications or patents of said patents; US 6,039,946 and US 5,753,227 US 6,264,623 and published US patent application US 2001/0039392A1, US 2002/0019603 A1 and all their US divisional applications and issued patents and all equivalents and foreign counterparts of said patents and the divisional patent applications and divisional patents of said patents. PCT WO96/37516 and its foreign and US counterparts and all divisional applications thereof. For abbreviation all these patents and patent applications will be referred to at times as "my patents". Also incorporated herein by reference are the following patents and publications , including the references cited in them: Nillson et al: US patent US 6251,394 B1, V Pimm: Nucl. Med . Biol. Vol. 22, No 2, pp. 1020-1027, 1993, D. A. Goodwin et al: Antibody Immunoconjugates and Radiopharmaceuticals, Vol. 4, number 4, pp. 427-434, 1991, Van Kroonenburgh et al. , Nucl. Med. Commun. , Vol. 9, pp. 919-930, 1988, M Gurkavij et al., Cancer Research (Suppl.) Vol. 55, pp. 5874s - 5880s, December 1, 1995.
The limitations inherent in methods known to date, for the targeting of ligands , such as Treatment Ligands (TL) and Visualization Ligands (VL) [The definition of these terms is in accordance with PCT application WO 96/37516 its US and foreign counterpart and their divisional applications.] , to a site in an organism, particularly , but not exclusively tumor site. As known in the art of targeting ligands to sites in the organism , such sites may be for example , an area of infarct in the heart , or a

ligands to sites in the organism , such sites may be for example , an area of infarct in the heart , or a specific organ such as the Thyroid gland ,as examples. The tumor sites , in accordance with the current invention are both solid tumors that are tumors originating from cells outside the blood and the bone marrow (e.g., Leukemias and Lymphomas) as well as "soft tissue tumors" such as Leukemias and Lymphomas. Examples of solid tumors are well known and include , for example, colon carcinoma ovarian carcinoma pancreatic carcinoma as well as sarcomas. , as well as brain tumors, such as Asrocytoma and Glioblastoma .The treated organism which is a mammal, including , but not limited to a human, is conceptualized in accordance with the present invention to contain three compartments: The Tumor Compartment (TC) , The Blood Compartment (BC) and the rest of the organism : The Healthy Tissue and Healthy Organs Compartment (HC), including for example , the liver , kidney, spleen and lymph nodes. It should be realized that some or all of the organs may include tumor cell masses (such as metastases of tumor) . As used in the current invention the non tumor tissue and cells of the organ (kidney and liver for example.) will be referred to as HC and the metastatic cells (typically in the form of multiple cells) : are referred to as TC, even though they reside in the kidney or the liver , for example. It is clearly appreciated that the three compartments are in fluid communications, through , but not limited to the interstitial fluid (IF) , the capillaries of the blood circulatory system and the fluids of the Nervous System , Cerebro Spinal Fluid (CSF) and peritoneal fluid (PF) , for example. Further more , there is also fluid communications between the intracellular fluid (ICF) and the other compartments , including the above fluid compartments,for example the IF. It is also realized , that various chemical and cellular species , such as proteins , peptides, various antigenic and haptenic molecules, whether endogenous to the organism , or administered to the organism (such as TL and VL) various sub populations of lymphoid cells and macrophages, invading microorganisms such as viruses, bacteria and protozoa, are able to move from one compartment to the other , and may be at a steady state balance between the various fluid compartments and TC, BC and HC, meaning that removal of a molecular or cellular species from the BC, may for example change the rate of movement of the removed species from the other compartments to the BC.

In accordance with the present invention the tumor cells of the present invention contain Tumor Antigens (TA) and the Blood Compartment (BC) may contain Circulatory Tumor Antigens (CA) . It will be realized in accordance with the discussion above , that the CA, being a chemical species , usually will be able to migrate between the various compartments. A CA may be identical in chemical and antigenic structure to TA or it may have chemical and antigenic structure that is similar to but not identical to TA (for example the CA may have different affinity to specific monoclonal antibodies specific to TA then the affinity of same antibodies to TA). The body of the organism may produce antibodies to the TA and /or the CA. This antibodies are produced by the organism, as the result of the immune system in the organism mounting a humoral immune response directed at the TA and /or the CA . This antibodies are referred to as Native Antibodies (NAB) . NAB may act as "Enhancing Antibodies" (K. A. Hellstrom and I. Hellstrom: In: Encyclopedia of Immunology, I. M. Roitt and P. J. Delves Eds. Academic Press , 1998 p. 2440-2445) , such antibodies may bind to TA and mask the TA on the tumor cells, they may bind to lymphoid cell receptors and inhibit their ability to kill tumor cells, or they may participate in inducing suppressor lymphoid cells that inhibit tumor killing by Cytotoxic T cells (CTL) Examples of TA are given in WO 96/37516 and include Carcino Embryonic Antigen (CEA), Le(y), Alpha-Fetoprotein (AFT). Many other tumor antigens which include tumor specific antigens and developmental antigens (for example ovarian carcinoma CA-125 antigen) as well as monoclonal antibodies specific to these antigens are known in the art. (B. J Van

den Eynde and A. M. Scott: in Encyclopedia of Immunology , Supra pp. 2424-2430 .) In the presence of both CA and NAB , at least some of the CA and NAB bind to each other and produced CA-NAB complex . Such complexes may be in "antigen excess" (CA2NAB) , wherein the complex will generally have free antigenic sites available to bind additional antibody molecules, or it may be in "antibody excess" (CANAB2) , wherein the complex will generally have free antibody binding sites available to bind additional antigen molecules. CA-NAB , was found to play a role in the etiology and pathogenesis of cancer (F. A. Salinas and M. G Hanna , Jr. Eds. Contemporary Topics in Immunobiology, Vol. 15 :Immune complexes and Human Cancer, Plenum Press , 1985) and removal of CA-NAB by plasma exchange can be therapeutic (Immune complexes and Plasma Exchange in Cancer Patients, B. Serrou and C. Rosenfeld , Elsvier/ North-Holland Biomedical Press , 1981)

Factors that interfere with targeting TAB-bound VL or TL to a tumor site in an organism include:

(1) Presence of CA or CA2-NAB, that competes with the TA for binding of the TAB.
(2) TAB(bound to VL/TL) or CA-TAB (bound to VL/TL) complex binds to receptors such as Fc Receptors. If TAB is toxic, for example , is radioactive or is a toxin or toxic drug, this will lead to increased concentration of toxic VL/TL in healthy organs such as the liver , by binding of the TAB's Fc to Fc receptors on liver cells, when TAB contains Fc , such as when TAB is an intact antibody (it should be realized as mentioned earlier that TAB as defined in the current application may also be an antibody fragment , including synthetic fragment and fragment produced by genetic engineering. Complexes of such fragments with CA , even though they do not have Fc fragment, and do not bind to Fc receptors in the liver, they can still be cleared in the RES in the liver and elsewhere in Reticulo Endothelial System (RES) , by RES cells, such as macrophages.
(3) NAB compete with TAB for binding to TA and may inhibit TH1 Helper Cells' immune response to the cancer.
(4) CA-NAB complex concentrates in the RES, liver and kidney , for example , within the HC and toxic effects on normal organs is induced by this concentration.
(5) Particularly , following repeated administration of heterologus targeting antibody (eg: mouse monoclonal targeting antibody to human recipient) , the recipient may produce antibodies specific to the targeting antibodies . Such antibodies may be specific to the Fc part of the antibody (antiisotypic antibodies) or they may be directed to the Fv fragment (antiidiotypic antibodies) Such anti-targeting antibody antibodies (ATAA) can be produced by the organism, also when the TAB is a chimeric or humanized monoclonal antibody (M. V. Pimm Supra , Van Kroonenburgh et al., Supra.) ATAA may compete with TA for the binding of TAB.

It should be realized that depending on the particular cancer and the individual case, CA, CA-NAB complex , NAB, free ATAA TAB-ATAA complex (after the administration of TAB) may be present in various concentrations in the BC HC and TC. Optimally, particularly when these various concentrations are not known , it may be advantageous to remove or otherwise reduce more then one of the above species, and at least in some situations it may be desirable to remove as many of these species , as possible from the BC and/or HC and/or TC, prior to the administration of the TAB-VL or TAB-TL. Reduction of the amount of one or more of the above species in the BC and/or HC and/or TC, prior to the administration of the TAB-VL or TAB-TL , can preferably be achieved , in accordance with the present invention by Extracorporeal Adsorption , but may include , in some situations , reduction of one or more of the above species, by the administration to the subject of agents that would increase the breakdown and /or clearance from the body compartments of one or

more of the above species, that are known to increase such clearance from the organism (see , for example, D. A. Goodwin, Supra).

One approach that was used to reduce this interference is the administration of unlabeled targeting antibody prior to the administration of the labeled targeting antibody. This approach was associated with limited and various success (see for examples: M Helma et al. Cancer Immunol. Immunother. (1998) 47: 39-46, Beatty, B. G. et al. Cancer Res. 49, 1587-1594, 1989., Ahonen et al.: Acta Oncologica (1993), 32, 7/8 , pp. 723-7., Schrijvers A. G. H. et al.: J Cancer Research, (1993) 53, 4383-O4390, September 15, 1993. Clearly significant improvement in specificity (e.g. : to target cell , target tissue or organ, relative to the rest of the body) of targeting is needed. Pre administration of unlabeled targeting antibody ,while binding to circulating antigen or to CA2NAB in the blood circulatory system (or in other biological fluid such as for example, cerebrospinal fluid (CSF) and peritoneal fluid) and thus reducing the amount of subsequently administered labeled antibody in the blood and /or in the liver , by reducing the amount of circulating antigen that can bind the labeled antibody, the pre administration of unlabeled antibody will also lead to the unlabeled antibody binding to the TUMOR ANTIGEN ON TUMOR CELLS and by this mechanism will REDUCE targeting to later administered labeled antibody to the tumor target .

Thus, generally Extracorporeal Adsorption (ECA) is preferred , in view of the fact that such a method is not associated with administration of additional species to the subject (that may have their own toxicity) and is not associated with increased concentration of TAB, TL or VL in healthy organs of the subject, associated with clearance, such as the liver and kidney. Generally the removal of one or more of the above species is completed 15 minutes to 48 hours prior to the administration of the TAB-VL or TAB-TL. It should be realized that the Targeting Ligand may be a species that is not an antibody species and its binding to the targeted site in the organism , may be based on none immunologic binding, as detailed and described in the incorporated references, in particular patens 6,039,946 and 5,753,227 and their counterparts and in Pimm , Supra, Gurkavich, Supra and Goodwin, Supra. Thus the targeting ligand may be a ligand that binds none immunologically to a receptor such as Epidermal Growth Factor Receptor, or it may be the peptide hormone Somatostatin , that binds to the Somatostatin Receptor , which is present in high concentration in many tumors. The targeting ligand can also be other targeting proteins or peptides (OTP) , or they can be none peptide targeting molecules such as a drug, for example Atenolol that binds to Beta-Adrenergic Receptor and Haloperidol, that binds the Dopamine 2 receptor

My US patents and patent applications Supra and in particular PCT applications WO 96/37516 provide for improved targeting over the previous art, by including a step of ECA of a species comprising circulating tumor antigen circulating tumor antigen-antibody complex and circulating (such as enhancing) antibodies specific to tumor antigen.

These methods will not be associated with competition of the administered unlabeled antibodies with binding of the later labeled antibodies to the tumor antigen at tumor sites . This methods will also not include the risks and undesirable effects of administering of unlabeled antibody to the organism, (infection , reaction to foreign protein Immune complex Disease). Optionally the methods of my patents Supra can also add a step of removing of the targeted treatment or diagnostic ligand by

extracorporeal affinity adorption at a predetermined time following the adsministration of the treatment ligand or visualization ligand to further improve targeting.
ECA removal of targeted ligands is disclosed in US patents 6,046,225 and 6,251,394 and PCT application WO 96/37516.

The removal of circulating immune complexes can be achieved in accordance with my patents supra by specific extracorporeal immunoadsorbents such as anticomplex antibodies , or by Protein A affinity adsorbents (Fresenius Immunosorba ®, Fresenius Prosorba ®, Fresenius C1q extracorporeal adsorbent Miro ®, Kaneka Selesorb ® as well as Asahi, as disclosed in US patent 4,627,915 as examples. Enhancing tumor antibodies can be removed for exmaple , using affinity adsorbents such as tumor antigen, Protein A Immunosorba ® , Prosorba ® , for example, Ciq , (Miro ® , for example) as well as use of the adsorbents as used in Selesorb ® and the above Asahi patent. Removal of circulating free tumor antigen (not complexed with antibody) can be achieved by the use of adsorbents such as specific antibody to tumor
antigen.

It will be realized that tumor targeting inhibitor factors (TIF) may exist in complexes containing more than two molecular species. Such complexes may be affinity labeled for adsorption or adsorbed in the ECA column, by an affinity label or affinity adsorbent specific to any of the components of the complex, or specific to epitopes that are specific to the complex. Examples of such complexes are: CA-TAB-ATAA and NAB-CA-TAB. For example, the complex CA-TAB-ATAA may be adsorbed by antibody to any of its three components, bound non-covalently (e.g., by ligand) to Protein A that is bound covalently to the matrix in the ECA column, or it may be adsorbed by Biotinylated antibody that is bound to Avidin in the Avidin ECA column. Similarly, the CA-TAB-ATAA can be affinity labeled for adsorption, by administering to the subject a Biotinylated antibody, specific to any of its three components, to enable its specific adsorption in the ECA Avidin column or affinity labeled by antibodies to any of the components of the complex (or antibodies to epitopes specific to the complex) and adsorbed by ECA on a Protein A column.

EXAMPLE 1
STEP ONE OF EXTRACORPOREAL REMOVAL OF CA-NAB, AND/OR NAB AND/OR ATAA PRIOR TO ADMINISTRATION OF TAB-VL OR TAB-TL

Protein A-Sepharose CL-4B obtained from Pharmacia LKB Biotechnology and swollen and washed in accordance with the instruction (Affinity Chromatography . Principles and Methods, Pharmacia Biotechnology Pub., 1991), The Protein A-Sepharose is packed in a column . Preferably the column used is the commercially available Immunosorba ® sold by Fresenius HemoCare Inc. Redmond, WA. The column requires the use of a plasma separator , as is well known and as recommended by the manufacturer: either a "centrifuge" type, such as Fresenius AS 104 cell separator, Cobe IBM 2997 or membrane type plasma separator, such as Kaneka Sulfox ® or Cobe TPE ®can be used to separate, on line, the patient's plasma from the cellular elements of blood. While the Immunosorba ® column is preferred in some applications , other Protein A Adsorption columns can be used instead. When regeneration is preferred a system including two Immunosorba ® columns with a Fresenius Automatic Regeneration unit , Citem 10 ® are used. When no regeneration of the adsorbent , column

is needed only a single Immunosorba ® column can be used. When it is preferable to use a disposable column , the column utilized for adsorption can be the Fresenius HemoCare Prosorba ® column in which the Protein A is covalently bound to a silica matrix. It should be realized that other Protein A columns can be used instead, using various biocompatible matrixes to which the Protein A is covalently bound. (Whenever Protein A is mentioned in this application the term includes , Peptide fragments of protein A , including synthetic peptides, that are all known in the art to be used as equivalents of Protein A). The matrixes used include natural polymers, such as cellulose and dextran , various synthetic polymers and copolymers , such as polyacrylamide , polystyrene and polyvinyl polystyrene copolymer , and silica and silicones . One suitable matrix is heparinized silicone described in D. R. Bennett et al. US patent 3,453,194.

The configuration of the matrix is also not limited to a particular form , examples of suitable forms are beads (in particular, spherical in shape) , fibrous matrixes ,macroporous matrixes and membranes , including hollow fibers. One possible device is that of a typical multi-hollow fiber filtration, dialysis or diafiltration design. Such a configuration consists of a bundle of hollow fibers encased in a tubular housing. The adsorbent is included in the outer space of the hollow fiber , as disclosed in US patent 5.753,194, see in particular column 8 , line 28 to line 68 and figs 2 and 3, the patent uses in the example a chelator as the adsorbent, but in the current invention the adsorbent may be Protein A, either in free form , bound to a matrix , particularly by covalent chemical binding .or alternatively the Protein A can be bound to the membrane, to its blood flow side to the side opposite to the blood flow or to both sides , rather then being incorporated in the outer space of the hollow fiber. The Protein A, in either free form , or matrix bound, can also be physically trapped in the membrane, (such as when the membrane is an unisotropic posysulfone membrane , for example the one produced by Amicon) When the Protein A is encapsulated it can be encapsulated in one of the microcapsules or macrocapsules as described in US patent 5,753,227 , in particular column 7 ,line 53-65, or the double encapsulation described by Markus et al. American Heart Journal, Vol. 110 No 1 ,part 1 , July 1985, pp. 30-39. In the practice of the invention adsorbents other then Protein A , or in some situations , in addition to Protein A , can be used , that can adsorb CA, CA-NAB, NAB or ATAA. These adsorbents include for example, TAB (that will bind CA , CA2NAB and ATAA.) Protein G, Ciq bound to anti-C1q antibody, covalently bound to matrix, C1g covalently bound to matrix (for example, Miro ® sold by Fresenius HemoCare) Dextran Sulfate (for example, Selsorb ® sold by Kaneka Corp.) and the adsorbent disclosed in Kuroda et al. US patent 4,627,915. Optionally , the Protein A Extracorporeal column 11 line 7. In the application of this method in accordance with the current invention an intact antibody , or an antibody fragment containing Fc fragment , and specific to a moleuclar species , desired to be removed , in accordance with the current invention, is bound to the protein A in the column by none covalent binding, following procedures known to those skilled in the art (see for example, E. Harlow and D Lane ; Antibodies , A Laboratory Mannual , Cold Spring Harbor Laboratory Pub. Pp. 411-522, 1988. and Affinity Chromatography, Principles and Methods, Pharmacia LKB Biotechnology Pub. # 18-1022-29,, pp. 47-52). In accordance with the present invention the antibodies bound to the protein A in the ECA column are one or more of the following specific antibodies (SA) : Antibody to TAB , antibody to CA (for example TAB) antibody to NAB, antibody to ATAA (e. g. anti idiotypic antibody), CA-NAB , NAB (anti idiotypic antibody) antibody to a tumor blocking factors and suppressor cells such as : TGF ß, p15E, TH2 T cell epitope.
In the EXAMPLE, the organism being treated is a human , the adsorption system used is the Fresenius system containing two Immunosorba ® columns and a Citem 10 ® regeneration unit. Each

column contains 62.5 ml of Protein A , which is covalently bound to a cross linked beaded Sepharose ® matrix. Plasma is first separated from blood cellular elements, using Fresenius AS 104 Cell Separator . The plasma flow rate in the Immunosorba ® column is approximately 20-35 ml/min (flow rates can vary , depending on the individual case and can be in a range from 5 to 50 ml/min, when Immunosorba ® column is used.

Depending on the individual case and the particular column used , persons with skill in the art can determine without undue experimentation the appropriate flow rate , in the individual case. Step One above can be completed preferably from 0.1 hours to 24 hours prior to the TAB-VL or TAB-TL administration. Most preferably it will be completed between 0.15 hours to 4 hours , prior to the TAB .administration. The length of step one is typically between 1 hour and 17 hours and more typically between 2 hours and 4 hours, depending on the individual case including , for example , age , weight, presence of pathological fluids in body cavities , such as peritoneum, interstitial space, pericardium and pleural cavity, as examples. It should be understood that while in the EXAMPLE, plasma is being treated, other bodily fluids can be treated, such as peritoneal fluid, lymph, cerebro spinal fluid, with access to these biological fluids achieved by methods that are well known in the art. When fluid of the Blood Circulatory System is accessed and treated , the fluid may be blood rather then plasma (see for example US patent 5,753,227, including use of encapsulated adsorbent , when blood is directly treated) . Following the adsorption STEP ONE, in the EXAMPLE, in STEP 2 , the TAB is administered intravenously. The TAB in the EXAMPLE is Hybrid of the two intact monoclonal antibodies Mab CHA-255 , specific to the hapten L-SCN-C6H4-CH2-EDTA and Mab ZCE-025 specific to CEA (C. Lollo et al. , Nuclear Medicine Communications, Vol. 15, pp. 483-491, 1994) The VL is 111 In-NBE-EDTA , which is bound none covalently to antibdy binding site of Mab CHA-255. The hybrid of Mab CHA-255 and Mab ZCE-025 is prepared by using the method described by Lollo et al. Supra, except that instead of the hybrid F(ab')2 , a hybrid of the intact antibodies (which is the preferred species used) is the hybrid utilized. Alternatively it is possible to use hybrid of one intact Mab with the Fab fragment of the second Mab thus including in the hybrid Fc piece of at least one antibody molecules , to enable binding to Protein A and Protein G , used as the adsorbent in the extracorporeal column . US patent 5,753,227, that is incorporated in this application by reference reads in column 10, line 51 to column 11 , line 8: "When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens. such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of rheumatoid arthritis. for example, the application of the analytical method of J. Goding et al.. J. Immunological Methods. vol. 20, 1978. pp. 241-53, to extracorporeal affinity adsorption in accordance with the present invention. is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al.. supra) is covalently bound to any of the matrixes described in the current invention. for example Sepharose 4B CL The antibody specific to the antigen. for example monoclonal anti-body specific to LDL is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part. and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the ECA column as described in the foregoing examples for the treatment of atherosclerosis, Clearly it is possible to use Protein G instead of Protein A in this system."

The adsorbent used in the CEA column may also be , Mab CHA-255 bound to Protein A or Protein G, through Fc of the Mab , EDTA that is covalently bound to the matrix , preferably through a spacer arm having a length of between 5-20 carbon atoms. Alternatively the EDTA can be conjugated to IgG that will bind to the Protein A through The IgG Fc. The EDTA will adsorb Free 111-In , released in the blood circulatory system. .

A modification of the method is to use liposome incorporation of VL in accordance with WO 96/37516. The incorporated ligand may be TAB-VL (For example, Mab ZCE-025-Mab CHA-255-NBE-EDTA-111 In) or it may be any other 111 In containing species, such as EDTA-111 In and target the liposome to the tumor , by binding to the wall of the liposome, covalently, or by ligand (non-covalently) Mab ZCE-025 , or its fragment that will target the liposome to the tumor.

EXAMPLE 2

Is identical to Example ONE above , except that the STEP 3 of ECA of TAB-bound 111 In NBE-EDTA- 111 In , or free 111 In is omitted.

EXAMPLE 3

Is identical to EXAMPLE 1 , except that prior to STEP 1 of ECA, in order to increase the affinity of the adsorbent Protein A , used in the EXAMPLE, monoclonal antibodies specific to the ATAA , (anti idiotypic antiboies to the FV binding site of ATAA), are administered to the treated subject . The production of monoclonal Anti Idiotypic antibodies to ATAA , are well known to those skilled in the art, as ATAA is a complete antigen . while use of monoclonal antibodies , preferably , chimeric or humanized antibodies are preferre, polyclonal antibodies can be used instead. Instead of administering anti ATAA antibodies , Cold TAB , e.g. TAB that is not bound to a VL or TL , can be administered , instead. In either case the production of ATAA-Anti-ATAA or ATAA-TAB complexes will increase their adsorption by Protein A, hence their effective removal by ECA. It is realized that should TAB be administered it will also inherently have affinity to and bind CA as well as CA2NAB.

EXAMPLE 4

Is identical to EXAMPLE 1 except that in order to enable the extracorporeal adsorption in STEP 1 of free circulating tumor antigen (CA) that are not bound to NAB and therefor can not be adsorbed by the Protein A used as adsorbent (as well as Protein G or C1Q, when these are used as adsorbents) as well as enable the more effective adsorption of ATAA, prior to step 1 of ECA , TAB (e. g. Antibodies specific to CA) is administered (intarvenously, intraperitoneally or by other route , depending on the individual case). TAB will also bind to ATAA (as the Antigen of ATAA) producing ATAA-TAB complex , thus enhancing the adsorption of the ATAA by the Protein A Protein G or C1q adsorbent in the ECA column. These TAB antibodies are preferably monoclonal antibodies , preferably chimeric or humanized antibodies, but can be polyclonal antibodies. The amount of antibody administered can be determined by those skilled in the art , depending on the individual case and will generally be between 0.1 Mg/Kg to 2 mg/Kg, Preferably 1 mg/Kg. The time interval between completion of this antibody administration and STEP 1 , is relatively short , in order to reduce to minimum the access of unlabeled TAB to the tumor, thus reducing the amount of unlabelled TAB bound to TA, and reducing the competition of unlabelled TAB , with the Labeled TAB . In view of

the fact that when unlabeled TAB is administered , such as by intravenous injection , it will have immediate access to CA in the BC , but a relatively delayed access to the tumor site , the time delay between completion of administration of unlabeled TAB and the initiation of STEP 1 ECA , will generally be between 5 minutes and 6 hours, preferably between 10 minutes and 2 hours. The time will depend on the individual case and can be determined by those skilled in the art without undue experimentation.

EXAMPLE 5

Is similar to EXAMPLES 1-4 , except that in order to enable adsorption of free NAB , by Protein A, Anti Idiotypic antibodies specific to NAB are administered prior to STEP 1 of ECA.

EXAMPLE 6

Is similar to EXAMPLES 1-5, except that in order to enable adsorption of tumor immunity molecular suppressors , (Such as TGFß and p15E) or tumor immunity cellular suppressors (such as TH2 suppressor cells), antibodies to the suppressor molecules and /or suppressor cells (e. g. In the example , antibodies to Th2 epitopes) is administered , preferably, prior to STEP 1 ECA.

EXAMPLES 7-12

Are similar to examples 1-6 except that the targeted ligand is a the TL Adriamycin , which is administered in accordance to EXAMPLE 2 of WO 96/37516, incorporated herein by reference.

EXAMPLES 13-19

Are similar to Examples 7-12 except that the Adriamycin is covalently bound to an intact Mab specific to the Tumor Antigen (TA) alpha-fetoprotein (AFP) and the method is in accordance with EXAMPLE 3 of WO 96/37516, incorporated herein by reference.

EXAMPLE 20 TO 26

The TAB is intact antibody ZCE-025 (see example 1) specific to CEA . It is directly Iodinated with 131 I , using the Chloramine-T (CT) method following the procedure of J. A. Carrasquillo et. al. Cancer Treatment Reports, Vol. 68, No 1, pp. 317-328, January 1984.

The TAB-131 I is administered in a dose containing 5 to 400 mCi radioactivity. The corresponding amount of Iodinated Mab is 0.65-52 mg. The other parameters of Tab administration are identical to those in EXAMPLES 1-6 In the above examples the 131 I TAB is used for TREATMENT. The dose used for diagnostics is 5 to 15 mCi . The procedures for adsorption of CA, and/or CA-NAB and/or NAB and/or TAAA and/or TGFß and/or p 15E and/or other tumor suppression factors are identical to those described in EXAMPLES 1-6.

In all the EXAMPLES 1-26, wherein prior to STEP 1 ECA, the treated organism is administered a species (TAB, Antibody to TAA, Antibody to NAB Antibody to a Tumor Suppression Factor , such as TGFß and p 15 E , for example) aimed at production of a complex between the administered species and a Targeting-Inhibitor Species or a Tumor Immunosuppressor species. Rather then administering the species to the treated subject , the species (such as the TAB , antibody to TAA etc. Supra) , which antibodies comprise an Fc piece (preferably the antibodies are intact monoclonal antibodies) The species is bound to the Protein A adsorbent in the ECA column, rather then being administered to the organism, following the general method of using ECA with Protein A or Protein G bound to a specific antibody , in accordance with US patent 5,753,227 incorporated by reference

EXAMPLES 27 to 52

The procedure for the adsorption of CA and/or CA-NAB and/or NAB and/or TAAA is identical to examples 1-26. The TAB is identical to the TAB of EXAMPLES 20-26, except that the TAB is treated in accordance to the procedures disclosed in US patent 6,251,394, for the labeling of the TAB for post TAB administration adsorption., This patent is incorporated herein in its entirety , by reference. The labeling of TAB is preferably with Biotin.

The TAB may contain any of the Therapeutic or Diagnostic ligands described in the above patent (as well as those described in the current patent application in its entirety, including , but not limited to EXAMPLES 1-26 above and those disclosed in PCT WO 96/37516 .).

The subject being treated for therapeutic or diagnostic purposes is a human subject with a malignant melanoma tumor which is positive for the p97 surface glycoprotein antigen. The TAB is the monoclonal antibody 96.5 specific to p97. The TAB is labeled with 131 I and conjugated to Biotin in accordance with US patent 6,251,394. See column 8 , line 66 to column 9 , line 19:

> "The monoclonal antibody was the 96.5 (mouse IgG2a) specific for p97, a cell surface glycoprotein with the molecular weight of 97,000 present on 60-80% of human melanoma. The tumor model has been described in detail (Ingvar. C. et al. Nucl. Med 30. 1989, 1224). 2. Conjugation and Labeling of Monoclonal Antibodies. The monoclonal antibody 96.5 (330 ug) was labeled with s 37 MBq iodine-125 (125 I), using the Chloramine-T method. By elusion on a Sephadex G25 column (Pharmacia PD10) the fraction containing the labeled protein was collected and used for the conjugation. The labeling efficiency of the 125 I 96.5 was around 70%. The radiolabelled monoclonal antibody was conjugated with biotin by mixing 500 ug of antibody with 41 ug of N-Hydroxysuccinimido-biotin (NHS-biotin) in 0.1M NaHCO3, O.15M NaCl with 10% DMSO. The mixture was incubated for 1 h at room temperature, followed by overnight incubation at 4° C. The 125 McAb-biotin conjugate was separated from free biotin-reagent by gelfiltration on a Sephadex G25 column, equilibrated with PBS (10 mM Sodium phosphate, 0.15 M NaCl, pH 7.3). The conjugate was stored at 4° C until used.

The dose administered to the subject is the same as in examples 20-26 (5-400 mCi, 0.65-52 mg TAB). 4 to 48 hours, preferably 12 to 24 hours after injection of the TAB , the subject is treated by passing his blood through an Avidin adsorption column, preferably Mitradep ® column produced by Mitra Products, Inc.

The length of adsorption is in most cases between 1 hour to and 10 hours , most preferably , between 2 hours and 4 hours and depends on the individual case , including body weight of the subject and dose of TAB, and can be determined by a person skilled in the art with out undue experimentation. The volume of plasma treated is between 1 and 6 plasma volumes , most preferably between 2 and 4 plasma volumes. The flow rate is between 10-50 ml/min. A scintillation camera is used for imaging , when imaging is desired."

In the step of removal of CA, CA-NAB, ATAA, NAB , TGFß, P15E and other molecular and cellular Tumor Suppression Factors (TSF) , rather then using Protein A as adsorbent, Protein A bound to a specific antibody to the TSF (TSF molecules and/or cells TH2 suppressor cells epitopes , for example), in accordance with the general ECA method , wherein the adsorbent is Protein A bound to a specific antibody, as disclosed in US patent 5,753,227, incorporated by reference. Protein A bound to specific antibody, can be used instead of, or in addition to free Protein A adsorbent (e. g. Protein A unbound to specific antibodies.) Alternatively to the above option, in the step of removal of CA, CA-NAB, ATAA, NAB and other molecular and cellular TSF , a FIRST affinity adsorbent, other then Protein A or Protein G, can be used for binding to the FIRST affinity adsorbent a SECOND affinity adsorbent, specific to the TSF , in accordance with the general principle of US patent 5,753,227, in particular column 10, line 51 to column 11, line 80:

> "When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens. such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of Rheumatoid arthritis. for example. the application of the analytical method of J. Goding et al.. J. Immunological Methods. Vol. 20, 1978. pp. 241-53. to extracorporeal affinity adsorption in accordance with the present invention. is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al.. supra) is covalently bound to any of the matrixes described in the current invention. for example Sepharose 4BCL. The antibody specific to the antigen for example monoclonal anti-body specific to LDL is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part, and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the extracorporeal immunoadsoption (affinity adsorption) treatment column as described in the foregoing examples, for the treatment of Atherosclerosis. Clearly it is possible to use Protein G Instead of Protein A in this system."

For example the FIRST adsorbent in the ECA column is Avidin or Strepavidin to which is bound a SECOND specific adsorbent , comprising Biotinylated antibody to TSF. The use of Avidin-Biotin

combination, wherein the FIRST adsorbent is Avidin, was proposed by J Tennval et al. Cancer Suppl. Vol. 80 , number 12, pp.2411-2418,December15,1997, for the removal of ATAA , by adsorbing to the Avidin in the ECA column Biotinylated TAB. In accordance with the current invention, this approach can be utilized to remove any molecular or cellular TSF , by binding to the Avidin in the ECA column one or more Biotinylated antibodies specific to one or more molecular or cellular TSF. It should be realized that the general method of US patent 5,753,227, utilizing a SECOND specific adsorbent , bound none covalently (e.g. bound by ligand) to a FIRST adsorbent that is covalently bound to a matrix, is not limited to Protein A-specific antibody or to Avidin-Biotinylated specific antibody pairs. Various pairs of affinity ligands can be used as the FIRST adsorbent and the SECOND adsorbent. The First adsorbent , for example, is an antigen or an hapten covalently bound to a matrix. For example, Dinitrophenyl (DNP) bound to a matrix, preferably through a spacer, or a carrier molecule, such as albumin (e.g. albumin-DNP conjugate bound to the matrix) . The SECOND adsorbent can then be a hybrid antibody specific to both DNP and the TSF. The first adsorbent can be an antibody to DNP and the SECOND adsorbent an antibody to TSF covalently bound to DNP. The FIRST adsorbent can be an enzyme bound covalently to the matrix in the ECA and the SECOND specific adsorbent will then be an enzyme substrate or enzyme inhibitor conjugated to an antibody specific to TSF (or to another molecular or cellular species targeted for removal.) Similarly any of other known affinity pairs such as , for example, those listed in US patent 6,251,394 , column 7 , lines 54 to 67, can be used. Such Specific Adsorption methods using a FIRST and SECOND adsorbents can be used not only in ECA treatment, but also in adsorption-based purification or diagnostic methods, to remove any molecular or cellular species from a fluid, including but not limited to biological fluids.

EXAMPLE 53-56

is identical to EXAMPLES 5-8 except that the TL is the anti cancer drug Calicheamicin. The subject treated is a human being having a Acute Myeloid Leukemia positive for the CD33 antigen. The TAB is Recombinant Engineered Human Anti-CD33. The TAB is conjugated to the Calicheamicin in accordance to L M. Hinman et al. , Cancer Research , Vol 53, pp. 336-3342, July 15, 1993. 3336-3342, The dose of administered TAB-Calciheamicin conjugate is 6-9 mg protein/ m2 (E L Sievers et al., Blood Vol. 93 (11), June 1 1999).

EXAMPLES 56-60

Are identical to EXAMPLES 1-8, except that the Targeting molecule , in these examples is a none immunologic OTP, the hormone peptide Somatostatin. The treated subject is a human being having a cancer with high concentration of Somatostatin Receptors, as determined by biopsy . (C. Casini Raggi et al. Clin. Cancer Res. Vol. 8 (2), PP. 419-427, Feb 8, 2002.) Adraimycin is conjugated to the Somatostatin in accordance with A. Nagy et al. Proc. Natl. Acad. Sci USA, Vol 95, pp. 1794-1799, 1998, The dose of the conjugate is calculated to contain 30mg to 75mg Adriamycin/ m2 body surface. The post administration of Somatostatin-Adriamycin conjugate clearence from BC and HC is done with an ECA column containing antibody to Somatostatin bound to Protein A. Alternatively , the Somatostatin is conjugated also to Biotin in accordance with C M eppler et al. J. biol Chem, Vol 26 7(22), pp. 15603-12, August 5, 1992 and the conjugate administered to the subject is Biotin-Somatostatin-Adriamycin and the ECA is done with an Avidin column , for

examole , Mitra ® ECA column. The subject is treated after post conjugate administration so as to treat 1-5 plasma volumes , ECA is started 1-48 hours after Biotin-Somatostatin-Adriamycin is administered , preferably 2-24 hours . The length of post conjugate administration is 1-4 hours. The flow through the column is 20-50 ml/min.

EXAMPLES 61-64

Are identical to EXAMPLES 56-60, except that the subject being treated is administered a Yttrium-90 labeled Somatostatin analog prepared according to A Otte et al. The lancet, Vol 351 , pp. 416-417, February 7, 1998. The dose of the conjugate is 25 mCi to 200 mCi.

EXMPLE 65

A conjugate of Avidin with Mab ZCE-025 ,which is specific to CEA antigen is prepared according to H. P. Kalofonos et. al. : The Journal of Nuclear Medicine , Vol. 31, No 11, pp. 1791-1796, 1990. , except that the Mab ZCE-025 is substituted for Mab HMFG1. The subject being treated is having a tumor positive to the CEA antigen and has circulating CEA antigen in his blood. As determined by standard methods known in the art and available commercially . In STEP 1: The subject is administered intravenously (1-4 min) 0.1 mg protein/Kg to 2 mg protein/Kg of the Mab-Avidin conjugate. In STEP 2: The subject is treated with ECA with a Biotin Adsorbent Column (BAC) . The BAC ECA starts 0.5h to 48 hours , preferably 2-24 hours after the administration of the Mab-Avidin conjugate. 1-4 volumes of plasma are treated . The length of the ECA is 1-4 hours . The flow rate is 20 ml/min to 50 ml/min. For its use as the adsorbent in the ECA Biotin is covalently bound to a macromolecular carrier, such as albumin , using the method described in US patent 6,251,394, except that the albumin is substituted for the antibody. Albumin labeling with Biotin can also be done , following the method described in Harlow and Lane Supra, pp. 340-341, substituting the HAS for antibody. The Albumin-Biotin is covalently bound to Cyanogen Bromide Sepharose 4B beads available from Pharmacia. As an alternative to binding of Albumin-Biotin to Cyanogen Bromide activated Sepharose , The binding of the Biotnylated Albumin to Sepharose can be done by using Avidin-Biotin binding The albumin is biotinylated , so as to have sufficient Biotin moieties conjugated to the Albumin ,thus leaving sufficient number of Biotin molecules in the ECA column to be available to bind to Avidin in the Mab-Avidin conjugate. In STEP 3: The subject is given intravenously 0.5-10 mg protein of the conjugate Biotin -Human Serum Albumin(HAS)-131 I in 1-5 ml volume of 8.4% Sodium bicarbonate. Human Serum Albumin is directly Iodinated with 131 I according to E. Harlow and D. Lane : Antibodies A Laboratory Manual, Cold Spring harbor Laboratory pub. , pp.324-329, except that Human Serum Albumin is substituted for the antibody for the direct Iodination. The preferred Iodination is by the Chloramine T method (E Harlow and D Lane Supra, 328-329.)

In STEP 4: 0.5-48 hours , preferably 2 hours to 24 hours following the administration of the Biotin-HSA-131 I- conjugate , the subject is treated by ECA column ,that includes Avidin as the adsorbent , Preferably the Avidin CEA column is the Mitra ® ECA column. Length of ECA is 1-4 hours, Flow rate in the ECA is 20-50 ml/min.

The purpose of Steps 1 and 2 is to remove CA , CA2-NAB and TAA from the BC and HC and from the Interstitial Fluid of TC ,by specifically labeling CA CA2-NAB and TAA , with the TAB-Avidin conjugate and adsorbing the complexes TAB-Avidin-CA TAB-Avidin-CA2-NAB and TAB-Avidin-TAA by adsorption to the Biotin adsorbent in the Biotin ECA column. The Mab in the TAB-Avidin conjugate can be an intact antibody , antibody fragment , including synthetic fragment, and fragment produced by genetic engineering techniques. In addition to the removal of CA, CA2NAB and TAA, removal of any other molecular and/or cellular species can be accomplished by the labeling of the species with a conjugate of Avidin that is conjugated to an antibody to the species, such suppressor species include : NAB, CA-NAB2 , Transforming Growth Factor beta (TGFß) p15E factor, Interleukin 10 (IL-10), Prostglandin E2 (PGE2), Mucin, Suppressive E Receptor (SER) , Immunosuppressive acidic protein (IAP) and adhesion molecules. (K E Hellstrom and I Hellstrom, Encyclopedia of Immunology Supra and C Botti et al. Int. J Biol. Markers, Vol. 13 (2) , pp. 51-69, 1998.).

The use of a labeled affinity targeting molecule can be utilized by administration to the subject being treated to affinity label any molecular or cellular species , in particular in the BC but also in the HC and the Interstitial fluid component of the TC, provided that the species targeted for affinity labeling is in equilibrium between the BC , HC and TC compartment (unless the removal is desired only from the BC or from the other treated biological fluid compartment , such as peritoneal fluid CSF or lymphatic fluid, when this fluid is treated in the ECA device, when equilibrium with HC and TC is not required. It should be realized that usually , these species will be in a concentration equilibrium between the various compartments). With respect to the species that it is desired to remove in the treatment (or diagnosis) of cancer, , in addition to what was listed above: Cellular species include TH2 suppressor T cells that suppress the immune destruction of tumor cells. It will be realized that the disclosed "labeling -based adsorption", will have applications other then in the treatment of cancer, to remove any endogenous or exogenously administered or invading cellular or molecular species , such as auto antibodies in the treatment of autoimmune disease, sepsis associated factors, such as Tumor Necrosis Factor , Leukotrienes, Bradykinin and Interleukin 2, in the treatment of sepsis. Viruses and bacteria as well as protozoa in the treatment of infectious diseases. Toxins : e.g. Tetanus toxin , Butullinum toxin, for example. Other utilizations include : Affinity labeling for ECA of specific cellular and/or molecular species that are collected or harvested for therapeutic use , in the same subject , from which they are removed , or in another subject: For example , removal of specific T cell population , for in vitro treatment followed by readministration to the subject , in the treatment of cancer , e.g. In vitro stimulation of harvested T cells by treatment with Lymphokines ,in LAK or TIL cell treatment of cancer (See for example J. J. Sussman et al., Ann Surg oncol, Vol 1 (4), pp. 296-306, 1994 and S A Rosenberg et al. , Science , Vol. 233(4770), pp. 1318-21, Sept 1986.) The only requirement for such affinity adsorption harvesting is the availability or the ability to produce specific affinity labeling reagent that is specific to the target molecular or cellular species , and the ability to conjugate the targeting species to an affinity marker , such as Avidin (or Biotin) , for example. (It would be obvious that the method can be modified , by for example, the use of Biotin for Affinity labeling and use of Avidin as adsorbent in the ECA, or the use of affinity pairs other then Avidin Biotin, for example : anti hapten antibody - hapten , Enzyme -substrate and the likes.
One significant advantage of the proposed affinity labeling - affinity adsorption (ECA) , is that it can be possible to use one single ECA device (Avidin-ECA, Biotin-ECA, for example) to remove many

different species form the subject, by using different specific affinity labels targeted to the species to be removed and adsorbing them on the single device used for the ECA step of the method. Altern The different species can be removed at different times or at the same time .

For example: If the species is TAA it can be targeted by TAB-Avidin or by Anti-idiotypic antibody to TAA and remove by a Biotin-ECA. If , the species is Oxidized LDL it can be affinity labeled by administering a conjugate of Avidin-Antibody to oxidized LDL and removed by ECA with Biotin as the adsorbent. Pairs of affinity labels and their counterparts are known in the art and are disclosed , for example, in US patent 6,251,394 , column 6 line 7 to column 8 line 53. Many specific ligands in the Avidin-specific ligand conjugate are known and many are available from commercial sources. Generally , such specific ligands are known by , or can be produced by persons with ordinary skill in the art, following established methods and without undue experimentation. For example , if the specific targeting ligand is a Mab, many of the molecular and cellular species , that are affinity targeted for removal have known Mabs that are specific to them and many of them are available commercially. For example Mabs to CEA and other tumor antigens mentioned in the current application. Alternatively , such Mabs can be produced, without undue experimentation by those skilled in the art, by use of Hybridoma Mab production techniques. As an alternative to administering a affinity labels to the subject , the TSF affinity labels can be incorporated in the ECA column , utilizing an ECA method that incorporates as adsorbents a FIRST and SECOND adsorbents. For example the FIRST adsorbent may be Avidin or Protein A covalently bound to the matrix in the ECA column and the "SECOND" adsorbent may be a pleurality of biotinylated antibodies (when Avidin is the FIRST adsorbent, or untreated antibodies (when Protein A is the FIRST adsorbent) that are specific to at least two TSF species. Use of Double Stage Labeling of a tumor for Radiolabeling a cancer was reported in H. P. Kalofonos et al. , The Journal of Nuclear Medicine , Vol. 31, no 11, pp. 1791-1796, Nov. 1990. The authors labeled a lung cancer with a conjugate of a Mab specific to the cancer antigen, that was conjugated to Strepavidin and administered Biotin- 111 In to radio-label the tumor for diagnostic imaging. The authors did not disclose or hinted to the possible use of such labeling for the ECA of molecular and cellular species . Matrixes other then Sepharose can be used to produce matrix-biotin conjugate for use in ECA. For example G. Paganelli et. al. Disclose the production of biotinilated Nitrocellulose and biotinilated Polystyrene (G. Paganelli et al. Int J. Cancer Suool 2, pp. 121-125, 1988.).

Avidin ECA column can be used with whole blood rather then plasma, thus simplifying the adsorbtion as disclosed by J. Tennvall et al. Cancer Vol 80, No 12 (suppl.) pp. 2411-2418 Dec 15, 1997.

Following ECA on the Biotin adsorbent Column, the subject is administered a VL or TL conjugated to Biotin (it should be realized that the use of Avidin and Biotin in the example can be reversed; with the sequence of : Step 1: Administration of Biotin-TAB, Step 2: ECA on Avidin column Step 3: Administration of TL-Avidin or VL-Avidin conjugate. Step 4 ECA on a Biotin column. It would also be realized that affinity pairs other then Avidin biotin can be used instead of Avidin- Biotin pair such as , for example: Hapten -Anti hapten antibody, Enzyme-substrate, Enzyme-Enzyme inhibitor.) The VL-Biotin conjugate can be Biotin- 111 In and is prepared and administered to the subject according to Kalofonos et al. Supra: Biotin covalently conjugated to Diethylenetriaminepentaacetic

acid (DPTA) is obtained from Sigma chemical comp. St. Louis, Mo and chelated to 111 In as described in Kalafonos et al. Supra.

Example 66

In STEP 1 , the subject is a human with CEA positive cancer , as described in Example 65 is administered a conjugate of Avidin with Mab ZCE-025 specific to CEA.

In STEP 2, The subject is treated with ECA , incorporating biotin as the adsorbent. The purpose of this step is to remove CA, C2 -NAB and TAA.

In STEP 3, An anti-cancer drug , including ,but not limited to one or more of the anticancer drugs disclosed in PCT WO 96/37516 as well as the anticancer drugs disclosed in US patent 5,527,528, incorporated herein by reference is incorporated in a liposome prepared as described in US patent 5,527,528 , in particular , example 3 , for the preparation of Avidin coated liposome omiting the last step of incubating the Avidin coated liposomes with biotinilated antibodies. Optionally the Avidin is bound to the Biotin on the liposome wall through a spacer. Suitable spacers are disclosed, for example , in K Hashimoto et al. Biochim Biophys Acta, Vol. 856 (3), pp. 556-65, April 25, 1986. The anticancer drug incorporated into the liposome in the Example is Adriamycin.
Step 4 , The subject is treated by ECA with Biotin incorporated as the adsorbent , to remove liposomes from the BC, that did not reach or attached to the cancer.

Optionally Step 2 , and/or step 3 can be omitted .

Alternatively the liposomes are Biotinilated liposomes of US patent 5,527,528, optionally with the Biotin connected to the liposome wall with a spacer arm, the Adsorbent in the ECA of Step 2 is Aivdin and the adsorbent in the ECA in step 4 is Biotin.

Example 67

The subject is the same as in example 66. STEP 1 he is administered Mab ZCE-025, specific to CEA conjugated to Biotin following the method of US patent 5,527,528. In STEP 2 , The subject is treated with ECA incorporating Avidin as the adsorbent. In Step 3 , the subject is administered Ricin A conjugated to Avidin (L. K. Mahal et al. Science , Vol 276, pp. 1125-1128, 16 May, 1997.)

In STEP 4. The subject is treated with ECA , incorporating Biotin as the adsorbent.

In all of the Examples wherein the administered species is Avidin conjugate of TAB, or the administered species is Avidin conjugate of a none immunologic targeting molecule, in order to remove from the treated subject , when desired any of the species that would inhibit targeting and/or species that would in general suppress the immune destruction , or none immune mechanism destruction of the tumor , such targeting inhibitors and/or tumor destruction inhibitors can be removed from the BC , HC and TC by incorporating in the ECA adsorption column one or more adsorbents that have specific affinity to the targeting inhibitor or tumor destruction suppressive

molecular and cellular species. This can be accomplished by incorporating such specific adsorbents in a single column, or in different columns , connected in parallel or in series , as disclosed in US patent 5,753,227. When the ECA incorporates Avidin as the adsorbent , The specific adsorbent added to the ECA Avidin column is a Biotin conjugate of a specific affinity ligand (such as Biotin-Mab specific to CA, and /or ATT and/or any of the other suppressors as disclosed in Hellsrom and Hellstrom, And in botti et al. Supra.) When the adsorbent in the ECA column is Biotin, the Specific adsorbent added is Avidin-Mab specific to the CA and/or ATT, or other targeted inhibitors or tumor destruction cellular or molecular species as above.

EXAMPLE 68

In any of the Examples 1-67, in the step of removal of specific species from the blood circulatory system or from other biological fluid, such as, peritoneal and CSF, the specific Protein A -Specific Intact antibody adsorbent ligand,can be used for the removal of any substance from a biological fluid source, and for the application of the current invention, can be used for the adsorption- removal of any chemical species that may interfere with targeting, such as CA, CA-NAB, NAB, ATAA, or any molecular or cellular species that suppresses the Immune Destruction, or None Immune destruction of the tumor. The specific adsorbent is based on the method disclosed in US patent 5,753,227. Said patent is incorporated in the current application in its entirety. In accordance with the current example, intact antibodies, or antibody fragments, containing Fc,, wherein said antibodies or Fc containing fragments are specific to an epitope on the molecular or cellular species that is to be removed from the biological fluid and BC, HC and TC are added to a Protein A extracorporeal column, such as Immunosorba ® or Prosorba ® , or any other Extracorporeal column that contains Protein A, bound to matrix or encapsulated, as disclosed for example in US patent 5,753,227, supra. Such antibodies or Fc containing fragments, in the current example are selected from antibodies, or fragments specific to the molecular and cellular species, that inhibit targeting or suppress tumor destruction as disclosed in the current application and in Hellstrom and hellstrom and Botti et al., supra.

As disclosed in US patent 5,753,227 and also detailed in Harlow and Lane Supra ,pp. 519-523, the antibody or fragment will bind to the protein A in the ECA through the Fc of the antibody or fragment, thus producing a specific adsorbent ligand to specifically adsorb one or more of the Targeting-inhibiting molecular species, or molecular or cellular species that inhibit or suppress tumor destruction. This can be accomplished by incorporating such specific absorbents in a single ECA column, or in different columns, connected in parallel, or in series, as disclosed in US patent 5,753,227.

APPENDIX B

Inventor: Meir Strahilevitz

EXTRACORPOREAL AFFINITY ADSORPTION AND OTHER AFFINITY BINDING TREATMENT METHODS AND DEVICES FOR ATHEROSCLEROSIS AND IN PARTICULAR CORONARY ARTERY DISEASE PARTICULARLY FOR UNSTABLE ANGINA AND OTHER ACUTE ISCHEMIC SYNDROMES

BACKGROUND OF THE INVENTION

It is well known that Coronary Artery Disease (CAD) and in particular its Acute Ischemic Syndromes (AIS) [Myocardial Infarction (MI) and Unstable Angina (UA)] are a major cause of morbidity and mortality. Revascularization Procedures (Coronary balloon Angioplasty, Angioplasty with Stent implantation and Coronary By Pass Surgery) are used when clinically indicated during AIS even though the associated morbidity and mortality is increased. Improved treatment of AIS, in particular UA, is highly desirable and acutely needed, in particular a treatment that can reverse some of the pathological processes in the Atheromas, thus enabling to stabilize the disease process, so that mortality from the disease episode is reduced and the patient can either improve without surgical intervention, or have sufficient stabilization to be able to undergo by-pass surgery or angioplasty, with reduced risk of morbidity and mortality associated with such procedures when they are performed without achieving sufficient level of stabilization of the pathological changes in the atheroma sites and as a result, in the patients clinical condition While lipid reducing medications can be associated with some regression in coronary atheromas, when used long term, they have little use in the setting of AIS. Extracorporeal Affinity and Extracorporeal Filtration methods aimed primarily at reduction of Low Density lipoproteins (LDL), while having a more pronounced and more immediate effect on atheroma pathology may still have limited use in AIS.

Extracorporeal Affinity Adsorption Methods aimed at removing from blood and body stores additional pathogenic chemical species, such as Oxidized LDL and other pathogenic chemical species as well as pathogenic cellular species may provide the needed non-surgical treatment that will meet the goal of inhibiting pathogenic processes in atheromas, resulting in sufficient quantitative changes within a short time frame, to enable pathological and clinical stabilization of patients with AIS, in particular UA, so as to either avoid the need for surgical

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or other invasive intervention, or alternatively, enable pathological and clinical stabilization, so that invasive revascularization procedures can be used with reduced morbidity and mortality.

The following published documents are incorporated herein by reference in the current application in their totality: US patents # 6,039,946 set out in Appendix A is attached to the current application, #5,753,227, and their parent application # 97,378, filed July 23, 1993 as well as its corresponding PCT application WO 95/03084 . Provisional US application 60/374715 (STRM 8363), set out in Appendix B is attached to the current application. The appendices are considered a part of this provisional application, and the references cited therein are incorporated by reference herein.

Numerous Molecular Inflammatory Factors (MIF) and Cellular Inflammatory Factors (CSF), (MCIF) have been identified as etilogical in the pathogenesis of Atherosclerosis (AS) : Including: Coronary Artery Disease (CAD), Peripheral Atherosclerotic Vascular Disease (PASVD), Atherosclerotic Cerebro Vascular Disease (ASCVD) . Included in CAD are Acute Ischemic Syndromes (AIS) that include Acute Myocardial Infarction (MI) and Unstable Angina (UA).

The MCIFs involved in the etiology and pathogenesis of Atherosclerosis (AS) and in particular UA, including those that are increased systematically, are summarized by V. Pasceiri and E. T. H. Yeh : Circulation 1999, Vol 100. Pp. 2124-2126. The molecular MCIF species increased in UA include Interleukin 6 (IL-6), IL-2 Receptor, C Reactive Protein (CRP) and TNF alpha . Cellular MCIFs include: CD3+DR+ lymphocytes, CD4+C28-(null) T cells, CD4+ INF gamma+ T cells, Th1 T cells.

Publications reporting the involvement of inflammation processes in AS and in particular AIS include: Z. Reiner et al. Lijec. Vjesn. 2001, Jan-Feb Vol . 123 (1-2) pp. 26-31. Found that Interleukin 2 (IL-2) was increased in UA and the level of IL 2 was correlated with the level of serum lipids. Increased levels of Metloproteinases and proteolytic enzymes may also be associated.Koukkunen et al . Annals. Medicine, Vol. 33 (1) pp. 37-47 reported increased C Reactive Protein (CRP), IL-6, TNF alpha, Fibrinogen, Troponin and Creatin Kinase in UA . K. K. Mizzia et al . reported that both TNF alpha pro inflammatory cytokine and IL-10, anti-inflammatory cytokine, were increased in both stable and unstable Angina. A. D. Simon et al.: J. Thrombosis and Thrombolysis, 2000, April, Vol 9. (3) pp. 217-22, found

increased IL1-beta and IL-6 in UA. W. H. Lee et al. : Exp. Mol. Med. Sept 30, 1999, Vol. 31(3) pp. 59-64, found increased level of CRP in plasma of patients with UA . M. Biasucci et al .: Circulation, 1999, April 27, Vol. 99 (16) pp. 2079-84, found that IL-6 and IL-1Ra were elevated in UA and that their level correlated with the likelihood of a complicated hospital course G. Caligiuri et al., Circulation, 2000 September 5, Vol 102 (10) pp. 1114-9, found that Oxidized LDL (OX-LDL) is an antigen that causes the specific proliferation of T cells of patients with UA, thus indicating that OX-LDL is involved in the etiology and pathogenesis (EP) of UA . A. Mazzone et al., Atherosclerosis, 1999, August, Vol. 145(2) pp. 369-74, found increase of IL- 6 in Acute Ischemic Syndromes (AIS) . S. Yazdani et al. American Heart J., 1998 August, Vol. 136 (2) pp.357-61, found data indicating that increased IL-6 is correlated with destabilizaion of atheroma in coronary arteries . H. Tashiro et al. Coronary Artery Disease, 1997 March, Vol. 8 (3-4), pp. 143-147, found that Macrophage Stimulating Factor MCSF is stimulating and Trasforming Growth Factor Beta is (TGF-Beta) is inhibiting the process of AS . D. A. Smith et al., W. H. Lee et al. Exp. Mol. Med., 1999, Sept 30, Vol. 31 930, pp. 159-64 found elevated CRP in MI and UA . M Hoffmeister et al. Am J. Cardiol. Reported elevated CRP in AIS. D. A. Smith et al. Circulation 2001, August 14, Vol. 104 (7) pp. 746-9, found that IL-10 has a protective role in AS. N. Nakajima et. al. Circulation 2001, Feb 5,Vol. 105 (5), pp. 570-75, reported elevated CRP in UA. K. A. Fox et al. Brit Med Bull, 2001, Vol 59, pp 69-87, pointed out that management of associated risk factors, such as hypercholesteolemia appears to have substantial benefits even during the acute in-hospital phase of Acute Angina. .E. Lindmark et al. JAMA 2001, Nov 7, Vol. 286 (17), pp. 2107-13, reported elevated IL-6, CRP and Fibrinogen in UA, particularly a strong independent effect of elevated IL-6 in association with increased mortality in UA.

J. Plutzky, Am. J. Cardiol., 2001, Oct 18, Vol. 88 (8A),pp. 10K -15K, reported elevated CRP, TNF alpha and IL-6. AIS. A. Bayes-Genis et. al. New England J. Med, 2001, Oct 4, Vol. 345 (14), pp. 1057-9 reported increased level of Pregnancy Associated Plasma Protein A (PAPP-A) in the plasma and in unstable plaques in AIS.

H. Lee et al., Int. J. Cardiol., 2001, Sept-Oct, Vol. 80 (2-3), pp. 135-42, reported increase in CD14 monocytes and HLA-DR+ T lymphocytes in the acute phase of CAD and specifically reported the CD14+ expression on monocytes and percentage of HLA-DR+ T cells

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are decreased during treatment and that the expression of CD14 represents the activation of monocytes during the acute phase of CAD . W. H. Lee et al. Cardiology, 1999, Vol. 92 (1), pp. 11-6, reported data indicating that the rupture of an atherosclerotic plaque and formation of thrombus may lead to activation of CD40 cells in platelets of patients with AIS.

G. Liuzzo et al., Circulation, 1999 Nov. 23, Vol. 100 (21), pp. 2135-9, reported an increase in number of CD4+CD28null T cells in UA . As discussed in Pasceri et al., supra, this increase in T cell population is associated with increased production of INF gamma, that stimulates macrophages to produce metaloproteinases and other proteinase enzymes. P. Aukrust et al., Circulation, 1999, Aug 10, vol 100 (6), pp. 614-20, reported that T cells positive for the CD40L soluble and membrane bound ligand on activated T cells and platelets, particularly in UA, may play a role in the triggering and maintenance of AIS. CD40L cells induced enhanced release of chemoatractant peptide from monocytes, a chemokin involved in the pathogenesis of AS. T. Nakajima et al. Circulation 2002, Feb 5, Vol. 105(5), pp. 570-75, reported increased level of CD4+CD28null (null) T cells in AIS . These cytotoxic cells were reported to efficiently kill endothelial cells In Vitro and the killing is increased by sensitizing the target cells by CRP. The authors found increased frequency of Perforin and CD16 expressing CD4+ T cells in the peripheral blood, as well as increased CD161 appearence on null cells after stimulation Perforin expressing CD4+ cells from UA patients were cytotoxic to endothelial cells In Vitro.

A. Mazzone et al. Atherosclerosis, 1999, Aug, Vol. 145(2), pp. 369-74 found increased IL-6 in AIS and also found that IL-10 had a protective role in AS. D. A. Smith et al., Circulation, 2001, Aug 14, Vol. 104 (7), pp. 746-9, reported increased IL-10 in Stable Angina and reduced IL-10 in UA, indicating a protective effect of IL-10 in AS.

G. Luizo et al, Circulation, 1999, Nov 23, Vol 100(21) pp. 2135-9 reported in UA increase in CD4+CD28null cells associated with INF gamma production that was associated with persistent antigenic stimuli. D. E. Newby and K. A. Fox Br Med Bull 2001, Vol 59, pp. 69-87, reported that use of antiinflamatory clopodogrel and glycoprotein IIb/IIIa receptor antagonists had positive effect on UA.

SUMMARY OF THE INVENTION

The improvement that is the subject matter of the current application is particularly aimed at providing Extracorporeal Adsorption Devices and Methods for the effective removal and reduction of MIFs and CIFs (together: MCIFs) species that are etiological in the pathogenesis of AS in particular AIS and most particularly in UA. As mentioned in the background section, the data indicating the implication of MCIFs in etiology and pathogenesis (EP) of AS and AIS are present not only in AS but also in other inflammatory and autoimmune disease (such as Rheumatoid Arthritis) and may be implicated in non-atherosclerotic vascular diseases such as those associated with autoimmune disease, thus the devices and the methods of the invention may be utilized in treatment of these diseases as well as AS in Cerebro Vascular Disease and Peripheral Vascular Disease. Utilization of the methods and devices can also be applicable to inflammatory diseases in general, including inflammation associated with infectious and some neoplastic diseases. MCIFs can be reduced not only by ECA affinity adsorption but also by increased metabolism and clearance, which can be achieved by passive immunization with specific antibodies and including the use of Catalytic Antibodies (C Tellier, Transfus Clin Biol, 2002 Jan Vol. 9, pp. 1-8) (including fragments, including synthetic fragments and analogs). Active immunization with immunogenic preparations of MIFs may have utilization in the treatment of more chronic aspects of AS, even though they would not have a role in the treatment of AIS. The MCIFs that are increased in AIS include the following: TNF alpha, Metaloproteinases, IL-6, soluble IL2 receptors, CD3+ DR+ T cells, CD4+CD28null T cells, CRP, INF gamma . Utilizing known methods for affinity removal of cells or using ECA affinity adsorption with specific adsorbents of MCIFs such as Mabs, including fragments, or other affinity ligands, such as receptors and synthetic receptors including synthetic receptor fragments and analogs, will lead to removal of MCIFs from the various compartments of the body including biological fluid compartments, such as blood, peritoneal and CSF for example. As well as and in particular from atherosclerotic plaques. The MCIF removal treatment may be combined with drug treatment that is currently used in AIS such as anti-inflammatory and fibrinolytic drugs, for example.

EXAMPLE 1: EXTRACORPOREAL REMOVAL OF IL-6 FROM PLASMA OR BLOOD

IL-6 is elevated in the blood and systematically in the body, as well as AS palaques (ASP), particularly in UA .The adsorbent used in the ECA is a Mab specific to IL-6 (L. J. Cornfield and M A Sills Eur J Pharmacol, Sept 4, 1991, Vol. 202(1) pp. 113-5. The Mab is incorporated in ECA device . Suitable devices and matrixes, including suitable methods for covalent and none covalent binding of the Mab to the matrix are those described in US patent 4,813,924 example 13 and fig 7.and US Patent 6,039,946 when modified for use for only one Mab adsorbent (it is noted that whenever Mab is mentioned, it includes fragments, including synthetic fragments and analogs.) The Mab can be encapsulated in a microcapsule or macrocapsule as described in US patent 6,039,946. See in particular patent 6,039,946 col. 8 line 31 to col. 11, l. 60. And fig 4. When Mab to IL-6 is encapsulated according to the original method of L Markus et al (Am Heart J Vol. 110(1), part 1, July 1985, pp. 30-39) either the modified macrosphere encapsulation as used in the patent, or the method as described in Markus et al can be used for the encapsulation of the IL-6 Mab. Preferably the Mab is covalently bound to cyanogen bromide activated cross linked Agarose as described in the patent except that Mab to IL-6 is substituted for the Mab specific to LDL.

Alternatively Mab to IL-6 can be bound in the ECA column by non-covalent binding to Protein A that is covalently bound to the matrix as disclosed in the patent. Alternatively the Avidin and Biotin columns disclosed and described in incorporated US provisional application 60/374715, for example, see examples 27-52 can be used to provide matrix-bound Mab, specific to IL-6. The Mab to IL-6 is used instead of the Mab 96.5 specific to P97 antigen disclosed in the patent application and the Biotinylated Mab to IL-6 is bound to Avidin in the Avidin ECA column, for example the Avidin column produced by Mitra Products (Mitradep® column) The biotinylated Mab is bound in the column by the high affinity ligand binding of the Biotinylated Mab to the Avidin. which is bound covalently to the matrix in the column. The plasma flow rate in the ECA device, is usually 20-35 ml/min (flow rate can vary, depending on the individual case and can be in the range of 5-50 ml/min. Depending on the individual case and the particular column used . Persons with skill in the art can determine without undue experimentation, the appropriate flow rate in the individual case.

The length of the each ECA treatment is typically between 1 hour and 17 hours and more typically between 2 hours and 4 hours, depending on the individual case, including, for example weight and age of the subject. The number and frequency of treatments will depend on the individual case and can be determined without undue experimentation, by persons with skill in the art. It will be understood that while in the Example, in the treatment of AS, Plasma (using a Plasma Separator on-line) or blood are treated, when the devices and the method of the invention are used for the treatment of diseases other then AS (Such as Autoimmune Disease, Rheumatoid Arthritis, Cancer and other Inflammatory Diseases any biological fluid can be treated. For example, Peritoneal Fluid and Cerebro Spinal Fluid. Methods to access such biological fluids are known in the art. However, in the treatment of AS, plasma and blood will be in the biological fluids treated.

EXAMPLE 2

Removal of multiple MIFs in the treatment of AS, in particular AIS, utilizes methods and devices disclosed in US patent 6,039,946, particularly as described in col. 8 line 31 to col. 13 line 62 and in Figures 4, 5 and 6. The methods and devices are essentially similar to Example One, except that multiple adsorbents are utilized, with each adsorbent being specific to or selective for each one of the MIFs . The adsorbents are incorporated in the ECA in accordance with patent 6,039,946. The preferred adsorbents are antibodies, preferably Mabs, including fragments . When an Avidin column is used, the Biotinylated antibodies are bound to the Avidin as described in Example 1, with respect to Mab specific to IL-6: The adsorbents incorporated in the ECA can be adsorbents for any of the MIFs, including but not limited to all the MIFs described in the background section of this application and to the species etiological in AS that are described in patent 6,039,946. Mabs are known to many of the MIFs and in any event can be produced by using techniques known in the art, for the production of Mabs.

The molecular species to be removed are selected from the following list, that intends to give examples and is not intended to limit the scope of the invention: IL-6, TNF alpha, Heat Shock Protein (HSP), antibodies to Oxidized LDL (OxLDL) antibodies to HSP, CRP, LDL, Triglycerides, IL-2, metaloproteinases, other proteinases, Fibrinogen, Creatine Kinase, IL-I - Beta, IL-I-Ra, PDGF, Angiotensin II, MCSF, Pregnancy associated plasma protein A (PAPPA).

The parameters of ECA procedure: Flow rate, length of treatment session, will be in the range of the parameters described in Example One.

Clearly, rather than antibodies, other affinity ligands can be used instead such as Receptors and their synthetic peptides which include the binding site for the molecule to be adsorbed, including syntheic analogs of receptors, other selective adsorbents, such as Dextran Sulfate or Heparin, or polyacrilic Acid, for example, to adsorb LDL, substrate of enzymes (Proteinases) or enzyme inhibitors, Chelators for removal of metal containing species such as Metaloproteinazes, as examples.

EXAMPLE 3

The treatment may be utilized by itself or in addition to ECA treatment as described in Examples 1 and 2. It involves the administration of antibodies, preferably Mabs, preferably chimeric or humanized and including fragments, synthetic fragments and analogs which are specific to one or more of the MIFs, for example the Mab specific to IL-6.

Also, in addition to ECA conventional ant inflammatory drugs, receptor antagonists and anti cogulants, for example and any other drugs used in the treatment of AS, in particular AIS, can be used in conjunction of the use of the ECA methods in all of the Examples of this application. In addition to conventional drugs, Anti Inflammatory Cytokines, such as IL-10, TGF-Beta (K Mizzia et al. Supra, M. Londei: In Encyclopedia of Immunology, I. M. Roitt and P. J. Delves Eds.,Academic Press 1992, pp. 443-445), can be administered in conjunction with the treatment described in Examples 1-5, or as a stand alone treatment.

EXAMPLE 4

This treatment is primarily for none acute stages of AS: The treatment utilizes Active Immunization with one or more of the MIFs administered in an immunogenic preparation.

The immunogenic MIF, is preferably administered in conjunction with an adjuvant, preferably an adjuvant known in the art o be suitable for administering to humans, for example, one of the adjuvants disclosed in US patent 6,054,127. The immunogen may be incorporated in a liposome in order to increase its immunogenicity, as known in the art (G. Gregoridas: Trends in Biotechnology, Vol. 13, pp. 527-37, 1995.) MIFs of MW below 10000 KD are

preferably conjugated to a suitable carrier, as known in the art, for example KLH, Albumin or peptides.

EXAMPLE 5

This method is used by itself, or in conjunction with any of the methods and devices described in Examples 1-4. The method involves the removal by affinity adsorption, of Cellular Inflammatory Factors (CIFs) . Optionally this is done by Extracorporeal treatment of, blood, plasma, or in some situations, other biological fluids, but in most applications of the method is48 done by removing blood and treating it outside the body, without the use of ECA, and returning the treated blood to the treated subject. Any one, or more of the CIFs mentioned in the background section can be removed.

The preferred CIF to be removed, is CD4+CD28null T lymphocytes, that are strongly implicated in the EP of AIS. In addition, or instead, other CIFs can be removed, including but not limited to the following: Th1 T cells, CD14 T lymphocytes, CD 40+ Platelets, CD8+ CD 116+ Cytotoxic T cells, CD11b+CD16+ Lymphocytes and CD56+ Lymphocytes. CIFs can be removed by affinity adsorption with specific antibodies, preferably Mabs and including fragments, as described in Provisional US patent application 60/374715.

CIFs can also be removed by affinity adsorption with Mabs, utilizing the method described in B. L. Levine et al. J Hematotherapy, 1998, Oct, Vol. 7 (5), pp. 437, utilizing Mabs specific to one or more CIFs to specifically remove them. The system described in the paper above utilizes magnetic separation with antibody coated magnetic beads and a MaxSep ® magnetic separator. Preferably, in the current Example, CIFs are removed using the Isolex 300 i ® system (Nexell Therapeutics), with the use of Mabs specific to the CIFs that are targeted for removal. The Isolex 300 i ® uses paramagnetic beads to which the specific Mab is bound, to remove the specific cells (CIFs), When it is desirable not to discard the CIFs, such as when it is desirable to collect them,, the Isolex 300 I ® system provides a unique peptide release system to separate the cells from the Mab bound magnetic beads. Depending on the CIF to be removed either the removal system of the Isolex 300 I ® or another suitable peptide can be used, or the cells can be separated by other means, such as change in pH, mechanical shaking, change in ionic strength, or use of soluble cell receptor (epitope) including synthetic fragments

of the receptor, including analogs. When in the Example the CIF targeted for removal is CD4+CD28null T cells, it is desirable not to reduce unnecessarily general immune function of the organism being treated. In order to accomplish this aim, first CD4+ cells (both CD4+CD28null and CD4+CD28+) are removed by the Isolex 300 I ® system, utilizing Mab specific to the CD4+ epitope. CD4+ cells are then eluted, as described above, The removed cells are then treated with beads that are bound to Mabs specific to the CD28 epitope, the bound cells are eluted : These are CD4+CD28+ T cells . These cells are returned to the subject being treated. The CD4+CD28null cells do not bind to the Mab-bound paramagnetic beads and are discarded, or collected for testing, if desired. Mabs specific to epitopes on CIFs can be produced by known hybridoma and other Mab production techniques, routinely used in the art. Many of the CIFs epitopes have known Mabs. For example, Mabs are known for CD28 epitope (S. Fretier et al., J Leukoc Biol, 2002, Feb, vol. 71(2), pp. 298-94, S. D. Singh and C. G. Booth, J Immunol. Methods 2002, Vol. 260 (1-2) pp. 149-56. Anti CD4 Mab is also known. B. Sawltki et al., Eu J Immunol, March 2002, Vol 32(3) pp. 800-9. CD4+ T Cells can also be removed by an Extracorporeal Adsorption System described by H. Onodera et al., Ther Apher, 1998, Feb 2(1), pp. 37-42.

APPENDIX A

US006039946A

United States Patent [19]

Strahilevitz

[11] Patent Number: 6,039,946

[45] Date of Patent: *Mar. 21, 2000

[54] EXTRACORPOREAL AFFINITY ADSORPTION DEVICES

[76] Inventor: Meir Strahilevitz, P O Box 190, Hansville, Wash. 98340

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: 08/464,898

[22] PCT Filed: Jul. 20, 1994

[86] PCT No.: PCT/US94/08043

§ 371 Date: Jun. 8, 1995

§ 102(e) Date: Jun. 8, 1995

[87] PCT Pub. No.: WO95/03084

PCT Pub. Date: Feb. 2, 1995

Related U.S. Application Data

[63] Continuation-in-part of application No 08/097,378, Jul. 23, 1993, Pat. No. 5,753,227.

[51] Int. Cl.[7] A61K 39/00; A61M 37/00

[52] U.S. Cl. 424/140.1; 210/630; 210/645; 210/646; 210/648; 210/651; 210/660; 210/661; 210/670; 210/688, 604/5; 604/6

[58] Field of Search 424/140.1, 210/638, 210/645, 646, 660, 661, 688, 648, 657, 670; 604/5, 6

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Attorney, Agent, or Firm—Polster, Lieder, Woodruff & Lucchesi

[57] ABSTRACT

Extracorporeal affinity adsorption devices which are aimed at the substantial removal of two or more compounds that are etiological in the pathogenesis of diseases in man, provide effective therapeutic intervention means for these diseases. The devices are particularly suitable for the treatment of atherosclerosis, cancer, degenerative and autoimmune diseases. Extracorporeal chelation and immunotherapy for artherosclerosis, extracorporeal chelation treatment with on-line regeneration or replacement of chelant, extracorporeal immunotherapy with antibody fragments, and extracorporeal immunoadsorption utilizing antibodies bound to Protein A are also disclosed.

67 Claims, 3 Drawing Sheets

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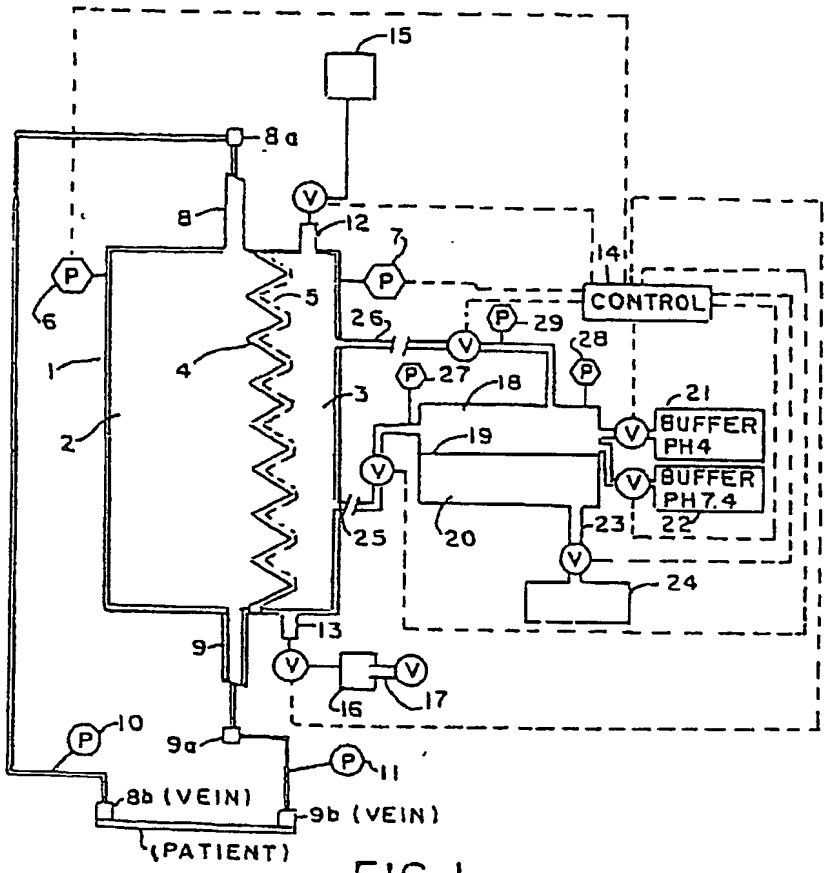


FIG. 1.

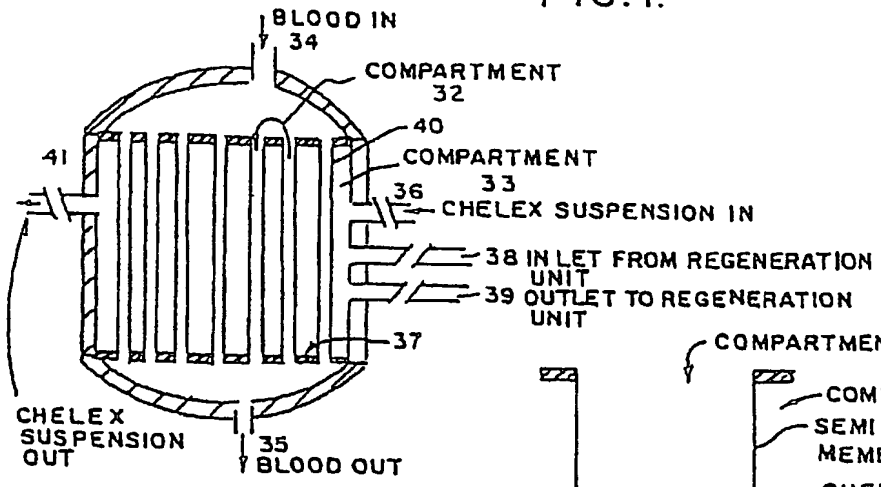


FIG. 2.

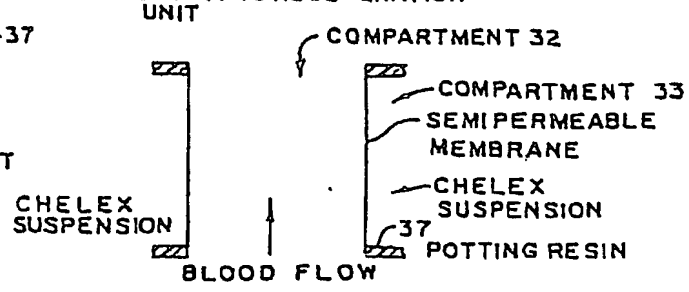
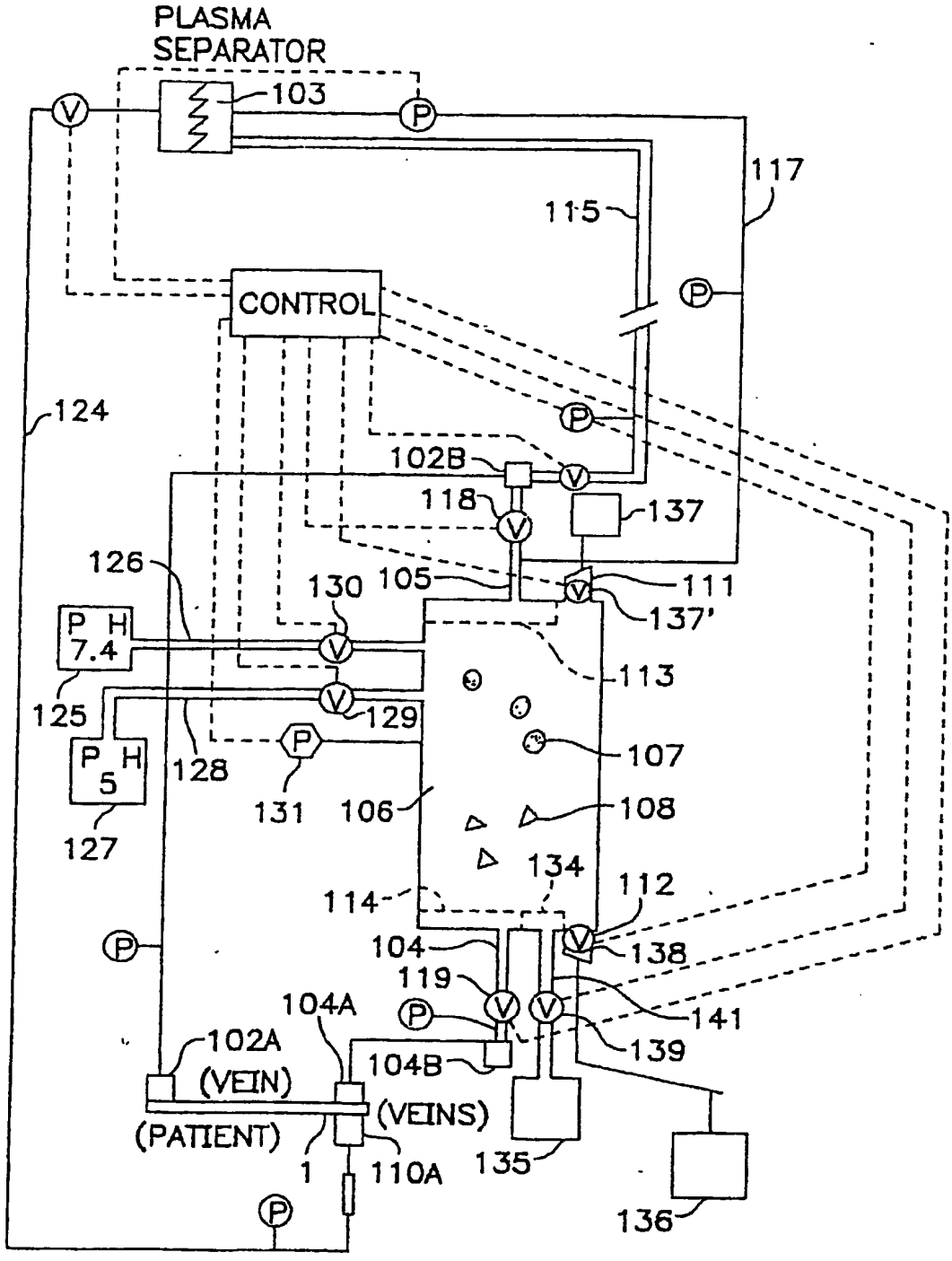


FIG. 3.


PLASMA
SEPARATOR
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106
108
127
134
112
114
104
138
119
141
104A
139
102A
(VEIN)
104B
(VEINS)
(PATIENT)
1
110A
135
136

FIG. 4

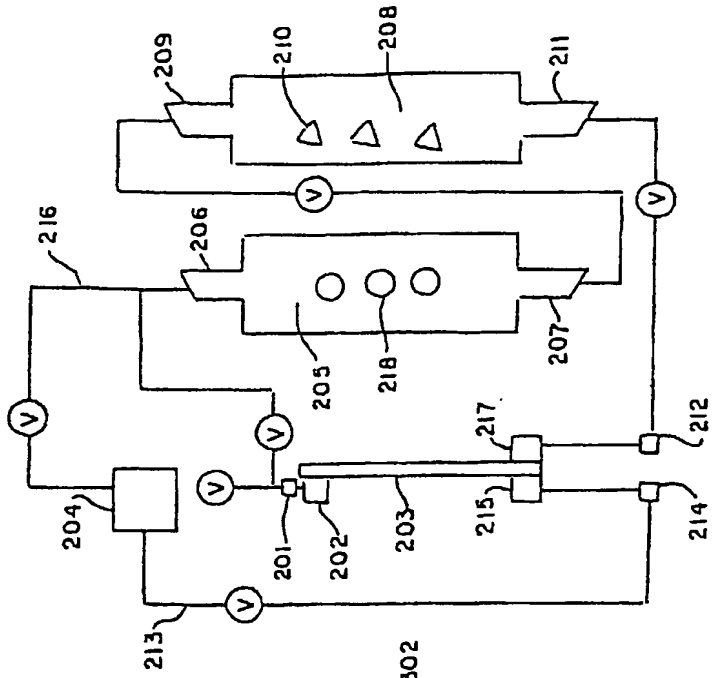
209
210
208
211
216
206
207
205
218
217
212
204
201
202
203
215
214
213

FIG.5.

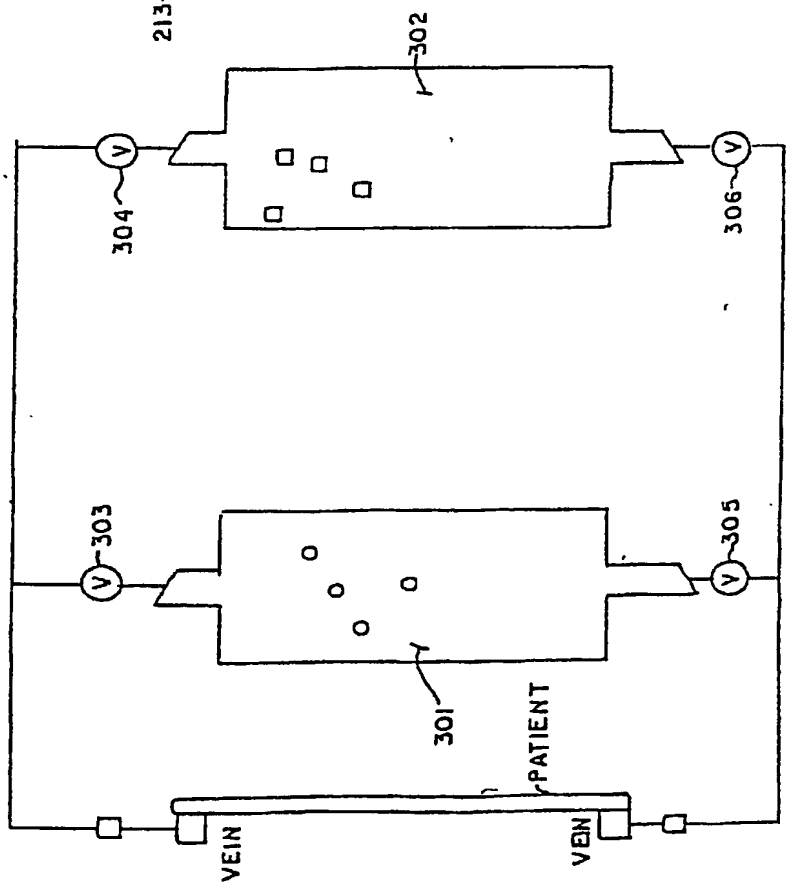
302
304
306
303
305
301
PATIENT
VEIN
VEIN

FIG.6.

EXTRACORPOREAL AFFINITY ADSORPTION DEVICES

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a 35 U S.C. 371 of PCT/US94/08043, filed Jul. 20, 1994, which is a continuation-in-part of U S Ser No 08/097,378, filed Jul. 23, 1993 and now U.S. Pat. No. 5,753,227

FIELD OF THE INVENTION

This invention relates to extracorporeal devices for binding substances in a fluid. The devices have particular but not exclusive application in the treatment of disease states wherein the fluid is drawn from and returned to a mammal.

BACKGROUND OF THE INVENTION

Atherosclerosis and cancer are the two major causes of morbidity and mortality in western societies. While there has been significant advance in the treatment of atherosclerosis there is still a great need for more effective treatment interventions.

The main mechanism by which atherosclerosis leads to morbidity and mortality is by narrowing the lumen of arteries and reducing the blood supply to the heart, brain and other vital organs. The factors associated with atherosclerosis include: High levels of cholesterol, triglycerides, low density lipoproteins (LDL) and low levels of high density lipoproteins (HDL).

Other factors are heredity, cigarette smoking, obesity, high blood pressure, reduced physical activity, high fat diets, and a high oxidation activity associated with the production of free radicals, leading to the oxidation of LDL, which accelerates the development of atherosclerotic lesions. Thus, J. Regnström et al., Lancet, Vol. 339, No 8803, May 16, 1992, pp 1183–86, reported that the susceptibility of LDL to in vitro oxidation in the presence of copper, which acts as a catalyst in the oxidation process, was correlated with the severity of their coronary artery sclerosis. J. T Salonen et al, Lancet, Vol. 339, No. 8798, Apr. 11, 1992, pp. 883–87, found that the level of autoantibodies to oxidized LDL predicted the progression of atherosclerosis of the carotid artery (the artery that supplies blood to the brain). One likely mechanism in development of atherosclerotic lesions via the oxidation of LDL is the induction of an autoimmune process leading to the production of antibodies specific to oxidized LDL and propagation of the atherosclerotic lesion by the autoantibody binding to oxidized LDL. J. Regnström et al, supra, and T. Kita et al., Proceedings of the National Academy of Sciences USA, Vol. 84, 1987, pp. 5928–31 J. T. Salonen et al., Circulation, Vol. 86(3), September 1992, pp. 803–11 reported an association between the risk of heart attack and the level of iron in the blood, with the risk being particularly high when plasma levels of both iron and LDL were elevated.

A significant reduction in blood levels of LDL and cholesterol by diet and lipid reducing drugs was found to result in regression of atherosclerosis. G. Brown et al., New England Journal of Medicine, Vol. 323(19), Nov. 8, 1990, pp. 1289–1298. Oral lipid lowering drugs, such as Lovastatin, MSD (Mevacor®, Merck), are risky and may cause liver damage. Their efficacy is relatively limited, even when they are taken in association with a strict diet. Lowering of LDL by extracorporeal treatment of blood, M. Strahilevitz, U.S. Pat. Nos. 4,375,414 and 4,813,924, and M. Strahilevitz, Atherosclerosis, Vol. 26, 1977, pp 373–77, is significantly more effective in reducing blood cholesterol and LDL levels H Borberg et al, Journal of Clinical Apheresis, Vol 4, 1988, pp. 59–65, R. L. Wingard et al, American Journal of Kidney Diseases, Vol. 18(5), 1991, pp 559–65; V Hombach et al., Dtsch Med. Wschr. Vol. 111(45), 1986, pp. 1709–15. LDL and cholesterol can be removed by affinity adsorption, utilizing as the adsorbent antibodies to LDL or other specific chemical adsorbents, such as dextran sulphate (M. Odaka et al., International Journal of Artificial Organs, Vol. 9, 1986, pp 343–48) or heparin (D. J. Lupien et al, Pediatric Res, Vol. 14, 1980, pp 113–17). LDL removal can also be achieved by heparin precipitation (D Seidel et al., Journal of Clinical Apheresis, Vol. 4, 1988, pp. 78–81), and by double filtration plasmapheresis (S Yokoyama et al., Arteriosclerosis, Vol. 5, Nov/Dec 1985, pp. 613–22) as well as by plasma exchange (G. R. Thompson, Lancet 1981 I, pp. 1246–48).

The oral administration of vitamin E is associated with lower risk of coronary heart disease in men (E. B. Rimm et al., New England Journal of Medicine, Vol 328(20), May 20, 1993, pp. 1450–56) and in middle aged women (M J Stampfer et al., New England Journal of Medicine, Vol. 328(20), May 20, 1993, pp. 1487–89). The mechanism of this protective effect is based on the antioxidant property of vitamin E, which inhibits the oxidation of LDL, thus exerting a protective effect from the development of atherosclerosis. The oxidation of LDL is catalyzed by heavy metals such as iron and copper. The removal of the metals by intravenous administration of chelating agents was reported to be effective in atherosclerotic vascular disease. E. Olszewer and J P. Carter, Medical Hypotheses, Vol 27(1), September 1988, pp. 41–49, and E. Cranton, "Bypassing Bypass," Hampton Road Publishers, Norfolk, Va., 1992. Others did not confirm these reports. S R. Wirebaugh and D R Gerates, DICP, Vol 24(1), January 1990, pp 22–24.

The apparent minimal effect, or lack of effect, of intravenous chelation in the treatment of atherosclerosis can be overcome by extracorporeal chelation which significantly increases chelation efficacy and reduces significantly its toxicity M Strahilevitz, Lancet, Vol. 340, Jul. 25, 1992, p 235

Extracorporeal chelation with desferoxamine was highly effective and safe in reducing blood iron in the treatment of hemochromatosis, a disease caused by the accumulation of excess iron in the blood and body stores. J. L. Held et al., Journal of American Academy of Dermatologists, Vol. 28, 1993, pp 253–54. Ambrus and Horvath in U S Pat. No 4,612,122 also describe a specific column configuration that can be used for extracorporeal chelation. In this column the chelating agent is physically immobilized in the spongy outer part of an anisotropic (asymmetrical) membrane.

Chelating agents can also be utilized with the extracorporeal affinity adsorption devices of Strahilevitz, U S. Pat. No 4,375,414

Coronary bypass surgery is effective in reducing symptomatology, but its effect on mortality is limited. J. H. O'Keefe, Jr. and B. D. McCallister, Editorial, Mayo Clinic Proceedings, Vol. 67, 1992, pp. 389–91, R. D. Simari et al., Mayo Clinic Proceedings, Vol. 67, April 1992, pp. 317–22.

Bypass surgery has no curative effect on the atherosclerotic disease process. The problem of post surgery atherosclerosis progression and the development of coronary or graft restenosis are major problems associated with bypass surgery. The need for effective means for reducing progression and inducing regression of atherosclerosis in patients

following bypass surgery is well recognized, as is the need to further develop effective nonsurgical treatments that would replace bypass surgery in a significant proportion of patients that are currently being treated with bypass surgery, because of the lack of alternative effective medical treatment.

This is particularly relevant for candidates for bypass coronary surgery with moderately severe coronary occlusion that may not exhibit significant fibrotic changes in the atherosclerotic coronary lesions. Similar limitations to those of bypass surgery apply to percutaneous transluminal coronary angioplasty. Simari et al., supra. In this procedure, an inflatable balloon is inserted into the coronary occlusion site. As with bypass surgery, this procedure also has no effect on the atherosclerotic disease process, thus restenosis is a significant problem. While the risks associated with angioplasty are lower than with bypass surgery, this is also an invasive procedure associated with morbidity and mortality risks.

While current medical treatments, particularly when combinations of conventional treatments are utilized, have significant effect in reducing progression and in inducing regression of the atherosclerotic process (Brown et al., supra), there is a need to have more effective treatment methods, particularly for those who can not be treated with oral lipid lowering drugs because of liver toxicity, who are unable to maintain a strict diet, or who fail to improve with conventional treatment, including oral lipid lowering drugs and diet.

The utilization of extracorporeal affinity adsorption of LDL (Strahilevitz, supra) can lead to marked reduction in LDL level, thus to significant regression of atherosclerotic coronary lesions Hombach et al., supra. However, the effect of affinity adsorption of LDL and cholesterol, while aimed at a major factor in atherosclerosis, hyperlipidemia, is selectively targeted on this factor. Even when (as usually is the case) the affinity LDL adsorption is utilized with other measures (diet, exercise etc.) the quantitative impact of these conventional treatment methods may not be sufficient. The availability of non-surgical methods that will have a significantly larger quantitative effect on additional factors that are involved in the etiology and pathogenesis of atherosclerosis is of great importance, in order to optimize the non-surgical and post-surgical treatment of atherosclerosis.

SUMMARY OF THE INVENTION

One of the objects of the present invention is to provide devices useful in effective non-surgical treatments of atherosclerosis.

Another object is to provide improved devices for extracorporeal treatment of atherosclerosis and other diseases.

Another object is to provide improved specific affinity devices, particularly immunoadsorption devices.

Other objects will become apparent to those skilled in the art in light of the following description.

In accordance with one aspect of the present invention, devices for treating atherosclerosis and other conditions are provided that are based on the utilization of specific affinity adsorption of several of the biological molecules that are etiological in the pathogenesis of the condition. The affinity adsorbents utilized in accordance with the present invention are both immunoadsorbents and non-immune-based specific affinity chemical adsorbents.

In some devices for extracorporeal combined treatment, one or both of the extracorporeal removals may be based on other principles than adsorption, for example use of extracorporeal double filtration for the removal of LDL. S Yokoyama et al., supra.

The adsorbents are incorporated in an extracorporeal treatment device The devices of the present invention will be usually utilized in conjunction with conventional treatment methods, both medical and, when indicated, surgical methods.

The novel treatment devices that are the subject of the present invention are based on and are specific improvements of extracorporeal affinity adsorption and extracorporeal affinity dialysis which are disclosed in Strahilevitz U S Pat. Nos. 4,375,414 and 4,813,924 and British provisional patent application No. 16001, May 20, 1971.

It is one of the objects of the present invention to provide additional specific improvements and embodiments to further increase the effectiveness and utility of extracorporeal affinity adsorption treatment of atherosclerosis.

Some of the elements of the present invention, as it applies to the treatment of atherosclerosis, are discussed in M. Strahilevitz, Lancet, Vol. 340, Jul. 25, 1992. p. 235, which states as follows (references omitted).

"Professor Salonen and colleagues' demonstration (April 11, p. 883) that titre of antibodies to oxidised low-density lipoprotein (LDL) is a predictor of the progression of carotid atherosclerosis should, among other things, encourage evaluation of treatment strategies that may reduce oxidised LDL. One such strategy you discuss in your accompanying editorial is the use of antioxidants, such as vitamin E. Another interesting possible therapeutic intervention to effectively reduce oxidised LDL in patients with severe hypercholesterolaemia and atherosclerotic disease is modified extracorporeal immunoadsorption of LDL. This affinity adsorption treatment, which uses specific immunoadsorption of LDL on columns containing antibodies to human apolipoprotein B in an extracorporeal circuit, is associated with striking reduction in LDL, clinical improvement, and regression of skin xanthomata and coronary stenosis.

"The procedure greatly reduces plasma LDL without much reducing HDL (one study reports an increase in HDL after the procedure). Although the therapeutic effect on atherosclerosis is assumed to be the result of selective removal of native LDL, there is no information about the possible reduction of oxidised LDL in the atherosclerotic lesion. Such a reduction could result from "desorption" of oxidised LDL from the atherosclerotic lesion into plasma, because of a substantial lowering of native LDL in plasma and the subsequent adsorption of the oxidised LDL to the LDL antibodies in the immunoadsorbent. Another mechanism that could lead to a decrease in oxidised LDL in the atherosclerotic lesion would be the reduction in production of oxidised LDL after substantial fall in native LDL in blood and body stores, including the atherosclerotic lesion. If the first mechanism operates, then it may be possible to increase the efficacy of extracorporeal immunoadsorption treatment by use of an immunoadsorbent that contains antibodies to oxidised LDL.

"You point out in your editorial that autoantibodies are probably not relevant to the initiation of the atherosclerotic lesion. Nonetheless, it is possible that removal of antibodies to oxidised LDL from plasma and body stores, including the atherosclerotic lesion, would be therapeutic. Such a goal can be achieved by incorporation of oxidised LDL, such as malondialdehyde-LDL in the extracorporeal immunoadsorption column. Alternatively, autoantibodies can be removed if protein A is used as the immunoadsorbent.

"A chelating agent adsorbent can be bound to the extracorporeal column matrix, to reduce oxidant levels in blood and body stores. With an extracorporeal device that incorporated desferrioxamine as a chelating adsorbent, serum iron level in a dog with an iron overload could reduced."

One aspect of the present invention is to provide novel devices for extracorporeal treatments for atherosclerosis based on specific affinity adsorption. The present invention also improves the efficacy of extracorporeal LDL affinity adsorption by combining it with affinity adsorption of ligands other than LDL and other lipids, that are also etiological in atherosclerosis.

Another aspect of the present invention is providing means for reducing the level of oxidized LDL in the body, using as affinity adsorbents specific antibodies to oxidized LDL, or using as specific adsorbent enzymatic digestion fragments of such antibodies, or synthetic fragments of such antibodies.

Yet another aspect of the invention is improving the immunoaffinity adsorption of LDL through the utilization of specific synthetic fragments of antibody (G. W Welling et al., Journal of Chromatography, Vol. 512, 1990. pp. 337–43), with synthetic fragments that are specific to LDL. Yet another aspect is providing means for extracorporeal affinity adsorption of autoantibodies to oxidized LDL, which may be etiological in atherosclerosis, by using as the specific adsorbent oxidized LDL (the antigen) such as malondialdehyde LDL (Salonen, Lancet, supra), or to use as the adsorbent of oxidized LDL autoantibodies, Staphylococcal Protein A (Strahilevitz, Lancet, supra). Rather than Staphylococcal Protein A, a recombinant Staphylococcal Protein A or Staphylococcal Protein A component, or other synthetic peptides of Staphylococcal Protein A may be utilized, as may Protein G or its components. Bensinger, U.S. Pat. No. 4,614,513; R. Lindmark et al., J. Immunological Methods, Vol. 62, 1983, p. 1. As used herein, except when the context clearly indicates otherwise, the terms "Protein A" and "Protein G" include all such variations.

When fragments of antibodies are used in the present invention as affinity adsorbents, they can be produced by enzymatic (e.g., papain or pepsin) digestion of the intact antibody to produce Fab, (Fab'2, or FV antigen-binding fragments, or they can be produced by other methods known to those skilled in the art for the synthesis of peptides, such as solid phase synthesis (R. A. Houghten, Proc. National Academy of Science USA, Vol. 82, August 1985, pp. 5131–35, R. E. Bird et al., Science, Vol. 242, 1988, pp 423–42) or through genetic engineering in a suitable vector such as *E. Coli* or phage (J W Larrick, Pharmacological Reviews, Vol. 41(4), 1989, pp. 539–57). The use of fragments, rather than intact antibodies, as the affinity adsorbent may increase the adsorption capacity and reduces side effects that may be associated with the Fc non-antigen binding part of the antibody molecule.

Another objective of the invention is to provide devices for extracorporeal chelation therapy for cancer, autoimmune diseases and degenerative diseases, such as rheumatoid arthritis.

An additional objective is to provide devices for extracorporeal combined treatment of cancer based on combining extracorporeal chelation and extracorporeal adsorption of enhancing tumor antibodies and their complexes by utilizing one or more of the following specific adsorbents, (a) Tumor specific antigen and (b) Staphylococcal Protein A or Protein G.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagrammatic view illustrating an affinity filtration device utilized for extracorporeal chelation therapy in accordance with the present invention.

FIG 2 is a multi-hollow fiber dialyzer or diafilter utilized for extracorporeal chelation therapy in accordance with the present invention

FIG. 3 is a detail of one hollow fiber of the device of FIG 2.

FIG 4 is a diagrammatic view of an extracorporeal affinity adsorption device for use with the present invention.

FIG. 5 is a diagrammatic view of two extracorporeal affinity adsorption devices connected in series for use in the present invention

FIG. 6 is a diagrammatic view of two extracorporeal affinity adsorption devices connected in parallel for use in the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following are examples of the preferred embodiments of devices and methods of the present invention. All of the examples utilize selective affinity binding of one ligand to another. The ligand which is held in an extracorporeal device will be referred to herein as a specific affinity "adsorbent," even in cases in which that ligand is in solution or suspension, and the process of binding a chemical species carried in a fluid by means of the specific affinity adsorbent will be referred to as "affinity adsorption."

Affinity Filtration Chelation

Referring now to the drawings, and in particular to FIG. 1, an apparatus is provided which corresponds to the apparatus of my U.S. Pat. No. 4,375,414, but in which a chelating agent is utilized as the specific affinity adsorbent. A column 1 is divided into a first compartment 2 and a second compartment 3, by a semipermeable membrane 4. Such membranes having various pore sizes and which are permeable to molecules of molecular weight below a particular weight only ("cut off") are available commercially. Preferred is a membrane with a pore size of 0.001 micron to 0 01 micron, a suitable molecular weight cut off is 1,000 to 10,000 daltons. One suitable membrane is a polysulphone membrane (M. Amato et al., The International Journal of Artificial Organs, Vol. 11 (3), 1988, pp. 175–80. Another suitable membrane is made of modified Cuprophan (Hemophan). (S K. Mujais and P. Ivanovich in "Replacement of Renal Function by Dialysis", Third Edition. Kluwer Academic Pub., J F. Maher Editor, 1989, pp. 181–98.

The membrane is preferably pleated to increase its surface area. The membrane is mechanically supported by a rigid mesh screen 5, facing compartment 3, thus avoiding contact of the mesh support material with the blood flow. Positive pressure pump 6 and negative pressure pump 7 are connected to compartments 2 and 3 and can be optionally operated when increased pressure across the membrane is needed for enhancing the mass transfer across the membrane between compartments 2 and 3 The overall surface area of the membrane is between 0.5 m^2–3 m^2. The pumps are connected to a control 14 to enable automatic operation Compartment 2 has an inlet 8 for a catheter 8*a*, which is to be connected to a vein 8*b* of the patient (when vein to vein connection is used, which is the preferred operation). Catheter 8*a* may however be connected to an artery of the patient when desired. Outlet 9 is connected to a catheter 9*a*, which is to be connected to another vein 9*b* of the patient. Together they comprise a blood flow passage through compartment 2. The second compartment 3 includes the chelating agent. Preferably the chelating agent is a multivalent chelating agent such as a biotechnology grade resin marketed by Biorad Corp. under the registered trademark Chelex 100.

This resin is a styrene divinylbenzene copolymer containing paired iminodiacetate ions, which act as chelating groups for binding polyvalent metal ions Chelation is based on coordination binding between the chelating agent and the heavy metals, which is similar to a covalent bond, but in which both electrons are donated by the same atom. Chelating binding differs from ion exchange by its high selectivity for heavy (transition) metal ions and by its much higher bond strength. Chelex 100 resin has the following selectivity for some cations (a higher number indicates greater affinity): Hg^{++}=1060, Cu^{++}=126, Na^{+}=1×10^{-7}.

The Chelex 100 resin is obtained in 200 mesh size and is ground to particles with a diameter of 5 to 30 microns. The dialysis fluid is a standard renal dialysis fluid, preferably bicarbonate type buffered to pH 7 4.

While Chelex 100 is preferred for some applications, other chelating agents can be used when bound to macromolecular organic or inorganic particles, such as triaminepenta acetic acid or deferoxamine bound to a suitable matrix, such as silica. Other chelating agents are derivatives of iminodiacetic acid such as EDTA and matrix bound glycine hydroxamic acid.

Peristaltic pumps 10 and 11 are optional and may be used as needed to accelerate blood flow into and out of compartment 2. The blood flow through the device is in the range of 25 ml to 250 ml per minute The system described thus far can be used without on-line regeneration, with the replacement of buffer and chelating agent as needed via inlet 12 and outlet 13 This mode of treatment can be operated manually or automatically, by the use of control 14 to operate the valving of inlet 12 and outlet 13, in conjunction with reservoir 15 that contains fresh Chelex dialysis fluid suspension. Reservoir 16 collects "used up" Chelex with its chelated heavy metals. Drain 17 can be used to clear reservoir 16, for discarding, chemical analysis or off line regeneration of the chelating agent.

Optionally, the system can operate with either manual or automatic on-line regeneration of the chelating agent. The automatic regeneration is identical to Strahilevitz, U.S. Pat. Nos. 4,375,414 and 4,813,924, except for the use of a buffer pH 4 rather than pH 2.5.

Because the chelating agent specifically binds heavy metals such as Cu^{++} and Fe^{++} that are mass transferred across the membrane barrier, a continuous gradient is present for the heavy metals that continue to mass transfer from compartment 2 to compartment 3 across membrane 4 by diffusion and/or convection, as long as compartment 3 contains free chelating groups that are available to bind heavy metals. When pressure filtration is utilized, by operating the optional positive pressure pump 6 and/or negative pressure pump 7, the process of mass transfer is accelerated by increased convection.

Optional means are provided for on-line continuous regeneration of the chelating agent. Preferably regeneration is achieved by use of a weak acid solution at pH 4 or higher. However, elution can also be achieved by use of a concentrated salt solution, or by the use of a low molecular weight free chelator, such as EDTA (specific elution). When on-line regeneration is used, the Chelex bound heavy metals buffer solution is transferred through outlet 25 to compartment 18 with optional operation of peristaltic pump 27. The buffer is pressure filtered through membrane 19 to compartment 20 (permeable to the buffer but not to the Chelex) The positive pressure for filtration is provided by pump 28. The elution (regeneration) buffer is then transferred from compartment 21 to compartment 18 and again positive pressure pump 28 transfers the heavy metals eluted from the Chelex to compartment 20 and through outlet 23 to compartment 24, from which it can be discarded or used for chemical analysis Buffer pH 7 4 is added from reservoir 22 to compartment 18 and the regenerated Chelex is returned to compartment 3 via conduit 26 Optional peristaltic pump 29 is used to accelerate transfer. Alternatively, other configurations can be used for on-line regeneration, such as the one utilized in S. P Halbert, U.S. Pat. No. 4,637,880.

While the method described thus far uses Chelex with beaded, preferably regular spherical, form, other forms of matrix can be used such as irregular beads or fibers, either natural or synthetic, to which the chelating moiety can be covalently bound or physically trapped (immobilized).

The chelating moiety can also be covalently bound to semipermeable membrane 4, when the membrane is made of synthetic polymer or from natural or modified polymer The binding can be to the membrane side facing the blood flow, the membrane side facing the dialysis chelator flow, or to both sides. The physical configuration of the matrix is not limited to any particular form, as long as the matrix configuration and its particle size prevents the chelating agent from being substantially transferred from compartment 3 to compartment 2.

The affinity chelation filtration can be utilized for affinity adsorption of a plurality of ligands, for example when free antibodies or antibodies bound covalently to a matrix or polymerized antibodies are included in compartment 3 and semipermeable membrane 4 is permeable to the antigen or hapten to which the antibodies are specific. An example for such antibodies are the antibodies to free cholesterol.

Affinity Adsorption Chelation

The configuration and process of this treatment is similar to affinity filtration chelation, except that the semipermeable membrane 4 and its mesh screen membrane support are excluded from the device of FIG. 1, along with pumps 6 and 7 A chelating agent, Chelex 100, ground to particle size of between 1–5 microns is encapsulated in a suitable microcapsule or macrocapsules. The macrocapsules used are those utilized by A. M. Wallace and A. Wood, Clinical Chimica Acta, Vol. 140..1984, pp. 203–12, for encapsulating antibodies and have an average diameter of 30 microns, or they are thermoplastic based macrocapsules (P. Aebischer et al., J. Biomech. Eng , Vol. 113 (2), 1991, pp. 178–83) having a diameter of 560±65 microns. Another suitable encapsulation of Chelex is by modification of the method of L. Marcus et al., American Heart Journal, Vol. 110, No. 1, Part 1, July 1985, pp. 30–39. The method used by Marcus et al. involved encapsulation of anti-digoxin antibodies in 0.2 micron polyacrolein microspheres, which are then encapsulated in 500–800 micron cross-linked agarose macrospheres. For encapsulation of Chelex, the Chelex is directly encapsulated in the cross-linked agarose macrospheres omitting the polyacrolein microencapsulation. Marcus et al. used their encapsulated antidigoxin antibodies in a column for extracorporeal adsorption treatment of digitalis intoxicated dogs. The same treatment was used in digitalis intoxicated humans. H. Savin et al., American Heart Journal, Vol. 113(5), May 1987, pp. 1078–84.

When Chelex is used in free form, particles of about 30 to 300 microns are preferred. Other matrixes and other chelating agents bound to the matrixes can be used. The matrixes can be of various chemical composition including, for example: natural polymers such as cellulose and dextran; various synthetic polymers and copolymers such as polyacrylamide, polystyrene, and polyvinyl polystyrene copolymer, and glass and silica. One suitable matrix is heparinized silicone described in D. R. Bennett et al., U.S.

Pat. No. 3,453,194 Various matrixes and methods for their activation for covalent ligand binding are described in P. D. G Dean et al., Editors "Affinity Chromatography· A Practical Approach," IRL Press, Oxford 1985, pp. 1–73. The configuration of the matrix is also not limited to a particular form; examples of suitable forms are beads (in particular, spherical in shape), fibrous matrixes, macroporous matrixes, and membranes including hollow fibers.

One possible configuration of the device is that of a typical multi-hollow fiber dialyzer or diafilter design. Such a design consists of a bundle of hollow fibers encased in a tubular housing.

In this configuration compartment 2 corresponds to the inner space of the hollow fibers and compartment 3 to the outer space of the hollow fibers.

In FIG. 2, blood flows from patient's vein through inlet 34 to compartment 32 (inner space of fibers). Heavy metals which pass across semipermeable membrane 40 are bound by chelation to Chelex suspended in compartment 33. The blood that flows out through outlet 35 is connected to another vein of the patient This blood is relatively free from heavy metals. Inlet 36 is used to replace used up Chelex suspension with new Chelex suspension. The old Chelex suspension is drained through drain 41. This step is optional. Optionally also, Chelex may be regenerated online by the same online regeneration means described with respect to FIG. 1. "Used up" Chelex suspension is transferred from compartment 33 through outlet 39 to the regeneration unit. Regenerated Chelex suspension is returned to compartment 33 from the regeneration unit through inlet 38. Suitable pumps may be utilized if it is desirable to increase the blood pressure in compartment 32 and across membrane 40, thus increasing the rate of mass transfer from compartment 32 to compartment 33 by a filtration process.

The space between fibers is sealed by sealing resin 37

FIG. 3 illustrates a single fiber in the unit. A typical fiber's membrane thickness is 6–30 microns. The combined inner membrane surface area is typically 0.75 to 1 2 meter^2.

Commercially available dialysers that can be used are Fresenius model F60 or Asahi PAN 150

Another embodiment of affinity adsorption device for use in the present invention is shown in FIG. 4. The device of FIG. 4 can be used to treat either blood or plasma. Particularly suitable for direct blood treatments are devices in which the matrix-bound chelator is encapsulated or when the matrix is a spiral structure such as for example natural polymer or synthetic polymer membrane to which the chelating moiety,is covalently bound. When plasma is treated in the device, a plasma separator is first used to separate on-line the patient's plasma from the cellular elements of blood. The physical configurations may include beads, in particular spherical beads, fibers, macroporous matrixes, membranes, and hollow fibers.

Blood may be directly treated, preferably when the matrix bound chelator is encapsulated. If plasma is treated, then the patient's blood flows via conduit 115 to plasma separator 103 (e.g., a centrifugal continuous plasma separator such as marketed by Cobe (Cobe IBM 2997) or preferably a membrane filtration plasma separator such as Kaneka Sulfox or Cobe TPE). The blood cells are returned to the patient via conduit 124 and the plasma is passed through conduit 117, via inlet 105 to column 106.

When encapsulation of matrix chelate is not utilized in the system and the method utilizes treatment of plasma, on-line manual or automatic regeneration can be used using a modification of the methods of Strahilevitz, U.S. Pat. No. 4,813,924 or the method of Halbert, U.S. Pat. No. 4,637,880.

In the on-line regeneration mode, with inlets 105 and 111 closed, outlet 112 to reservoir 136 closed, and either valve 119 or valve 139 closed, valve 130 is opened and buffer pH 7.4 is transferred from reservoir 125 to column 106. This is an optional step utilized when it is desirable to wash some of the patient's plasma that is present in column 106 into the patient's circulation (with valve 119 open) or to drain this washed volume of plasma through drain 141. This is done when it is desirable to reduce the amount of plasma proteins that is exposed to the eluting buffer. A small volume of buffer pH 7 4 is used, in order to minimize the volume of buffer introduced into the patient, when the option of returning the plasma to the patient is used. Alternatively the removal of plasma from column 106 back to the patient, can be accelerated by using positive pressure filtration with operation of pump 131.

In the elution step, with all valves except valves 129 and 139 closed, eluting buffer pH 5 is transferred to column 106 from reservoir 127; after equilibration, valve 129 is closed. valve 139 is opened and the buffer, including the free heavy metal cations passes through drain 141 Optionally, pressure filtration can be utilized with operation of pump 131. Optional filter 134 is permeable to buffer, heavy metal cations, and plasma proteins about the size of LDL, but not to Chelex and plasma proteins larger than LDL; the drained fluid is then collected in reservoir 135 and can be discarded or used for chemical analysis. In the next step column 106 is equilibrated with buffer pH 7 4, transferred from reservoir 125 through conduit 126.

When on-line regeneration is not used, replacement of used adsorbent by fresh adsorbent can be done manually, or automatically by automatic control of valves 137 and 138 with addition of fresh adsorbent from reservoir 137 and collecting used adsorbent in reservoir 136.

Column 106 is then ready for re-use Preferably vein to vein catheterisation is used, but when needed artery to vein catheterisation is utilized. When needed penstaltic pumps are used to accelerate fluid and mass transfer across the conduits 104, 105, 115, 117, 124 and 141.

The affinity adsorption device and method is well adapted to the concurrent adsorption of a plurality of ligands. On-line regeneration can be used when needed, and is particularly simple when the regeneration of the various adsorption ligates can be regenerated by the same regeneration means, such as by an acidic pH buffer, for example. The various adsorbents can be present in free form or can be encapsulated in microcapsules. Free form adsorbents are preferable because of their mechanical strength and suitability for regeneration, when desired. Encapsulated adsorbents will generally not be suitable for regeneration.

In FIG. 4, column 106 contains a first adsorbent 107 and a second adsorbent 108 Illustratively, the first adsorbent 107 is Chelex 100 in bead form, with a bead diameter in the range of 5 to 30 microns when encapsulated and 30 to 300 microns when free. The bead is preferably in free form, but can be encapsulated as previously described. The microcapsule membrane, when present, is permeable to heavy metals but not to Chelex or to plasma proteins. The Chelex specifically adsorbs heavy (transition) metals which catalyze oxidation of LDL. The second adsorbent 108 is cyanogen bromide activated cross-linked agarose (Sepharose, Pharmacia Fine Chemicals), with a bead diameter in the range of 212–300 microns, prepared according to R. E. Ostlund, Jr., Artificial Organs, Vol. 11(5), 1987, pp. 366–74 The Sepharose is covalently bound to monoclonal antibodies to LDL. (R. L. Wingard et al., supra) According to Ostlund, supra, LDL is adsorbed by the antibodies. As previously

described, rather than intact antibodies, antibody fragments can be used. The combined effects of significant reductions of both oxidation and LDL levels have a major impact on the atherosclerotic process.

Plasma which flows from column 106 though outlet 104 is substantially free of the species sought to be removed. Drains 111 and 112 can be used as needed for the removal of buffer and binding species, and for the addition of fresh binding species. Used binding species (e.g., anti-LDL antibodies and Chelex) can be regenerated off line, if needed. It should also be recognized that antibodies or fragments, "humanized" or hybrid antibodies (or fragments) can be used rather than mouse antibodies. J. W. Larrick, supra. In synthesizing antibody fragments, solid phase peptide synthesis methods (R. A. Houghten, supra) or genetic engineering methods (R. E. Bird et al., supra) can be utilized.

The advantage of Ab fragments over intact antibody is the reduced likelihood of side effects of the immunoadsorption treatment, particularly when whole blood is used for adsorption and the antibody or fragment is not encapsulated thus enabling contact of the mouse antibody with the patient's immune cells.

Additional adsorbents that can be utilized in the treatment of atherosclerosis include oxidized LDL, which will adsorb autoantibodies to oxidized LDL (cf. Salonen et al., Lancet, supra; Strahilevitz, Lancet, supra). Instead of the oxidized LDL, autoantibodies to oxidized LDL and their complexes can be adsorbed by use of Sepharose 4BCL Protein A, sold by Pharmacia Fine Chemicals. When Protein A is used as the adsorbent, the patient may need administration of replacement human gamma globulin. Additionally it may be desirable to adsorb oxidized LDL by using matrix bound antibodies to oxidized LDL as the adsorbent.

When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens, such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of rheumatoid arthritis, for example, the application of the analytical method of J. Goding et al., J. Immunological Methods, Vol. 20, 1978, pp. 241–53, to extracorporeal affinity adsorption in accordance with the present invention, is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al., supra) is covalently bound to any of the matrixes described in the current invention, for example Sepharose 4BCL. The antibody specific to the antigen, for example monoclonal antibody specific to LDL, is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part, and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the extracorporeal immunoadsorption (affinity adsorption) treatment column as described in the foregoing examples, for the treatment of atherosclerosis. Clearly it is possible to use Protein G instead of Protein A in this system.

When larger beads of cross linked Sepharose are used as matrix, they are prepared according to Ostlund, supra.

In the treatment of cancer the affinity adsorbents can include for example: Chelex 100 to reduce oxidation and Staphylococcal Protein A, or tumor specific antigens to remove enhancing tumor antibodies and their complexes.

An additional component of the combined treatment is to administer a radioactive drug or conventional drug conjugated to an antibody specific to a tumor antigen (such as Adriamycin conjugated to an antibody to Human-Alpha-Fetoprotein, R. Yang et al., Antibody, Immunoconjugates and Radiopharmaceuticals, Vol. 5, 1992, pp. 201–07), in conjunction with adsorption of the antibody-drug conjugate from blood. K. Norrgren et al., Antibody Immunoconjugates and Radiopharmaceuticals, Vol. 4(4), 1991, pp. 907–14.

The utilization of tumor-targeted radiolabeled antibody in conjunction with immunoadsorption of the radiolabeled antibody from the circulation to improve tumor imaging was reported by J. L. Lear et al., Radiology, Vol. 179, 1991, pp. 509–12. The adsorbent they used was an antibody to the radiolabeled anti-tumor antibody. The adsorbing antibody was utilized in an extracorporeal column in which it was covalently bound to a matrix. C. Hartmann et al., Journal of Pharmacokinetics and Biopharmaceutics, Vol. 19(4), 1991, pp. 385–403, evaluated the removal of radiolabeled antibody by extracorporeal adsorption, also using antibody to the radiolabeled antibody as the adsorbent. They found that the method would be effective for enhancing tumor imaging and for increasing the efficacy and reducing the toxicity of antibody-targeted anti-tumor drugs. These authors also cite two additional groups reporting similar results.

In accordance with the present invention, the anti-tumor drug or radiolabeled anti-tumor antibody is adsorbed in an extracorporeal column utilizing Staphylococcal Protein A as the adsorbent. This is a simpler and less expensive adsorbent and has the additional advantage of adsorbing enhancing antibodies and immune complexes; this enhanced removal has an important therapeutic effect on cancer. As previously mentioned, when Protein A is used as the affinity adsorbent, it may be necessary to administer intravenously, to the subject being treated, plasma or a plasma constituent such as gamma globulin.

It should be clearly understood that in enhancing tumor imaging utilizing antibody-targeted radioactive ligand, as disclosed in Hartmann et al., supra, Lear et al., supra, or Norrgren et al., supra, Protein A or Protein G can be utilized as the adsorbents.

Moreover, the radioactive imaging ligand may be incorporated in a hapten or antigen, preferably conjugated to the targeting antibody (or antibody fragment) by a spacer arm. The affinity adsorbent may then be an antibody to the free hapten or antigen, and all of the methods discussed above for binding drugs bound to targeting antibodies may be utilized. Engineered targeting antibody fragments are disclosed in D. J. King et al., Antibody, Immunoconjugates, and Radiopharmaceuticals, Vol. 5(2), 1992, pp. 159–70.

In the treatment of cancer the adsorption treatment will also be combined with conventional therapy such as chemotherapy.

Circulating immune complexes can also be adsorbed by C1q subcomponent of complement bound to specific antibodies to C1q, which are covalently bound to the matrix. T. Bratt and S. Ohlson, J. Clin. Lab. Immunol., Vol. 27, 1988, pp. 191–95.

In combined treatment of degenerative diseases (such as rheumatoid arthritis, for example) the adsorbents include Chelex 100 and Staphylococcal Protein A, or matrix immobilized human IgG to bind the rheumatoid factor which is an autoantibody to IgG.

When whole blood is treated in the column, the optional plasma separation system is bypassed and the blood flows from vein 102A directly to column 106 via inlet 105. Optional membrane 113 and membrane 114 are permeable to blood cells and plasma, but not to adsorbent-bound matrix, which in this application when used in particle form utilizes particles in the range of 300–800 microns in diam-

eter to ensure free flow of blood cells. The matrix can be in various other configurations such as fibers, membrane, capillaries, open porosity cavernous structure and the like The matrix can be made of blood compatible synthetic polymer, natural polymer and silica as examples. The filter 134 may be made of smaller pore size when molecules smaller than LDL, such as free cholesterol, are to be removed. When LDL is removed, filter 134 is permeable to molecules the size of LDL but not larger molecules.

FIG. 5 illustrates the use of two or more devices, either filtration adsorption or direct adsorption when each of the specific adsorbents is contained in its own column. The devices and treatment process can be operated manually or automatically. One or more of the devices can be regenerated on line or off line. Either whole blood or plasma is adsorbed Pumps as needed are included in the system to optimize fluid flow through the system. Pumps are also utilized as needed to increase trans-membrane pressure, when the filtration adsorption process is used

Referring to FIG. 5, a catheter 201 is inserted in vein 202 of patient 203, optionally passed through continuous plasma cell separator 204 that is of either centrifugal or membrane type The fluid (blood or plasma) is introduced into column 205 through inlet 206. Heavy metals in the fluid are adsorbed to Chelex 100 beads 218. The fluid leaves column 205 via outlet 207 It has a significantly reduced content of heavy metals such as Fe^{++} and Cu^{++}. The fluid is then introduced to column 208 via inlet 209. IgG and antibodies as well as antibody complexes are adsorbed on beads of Sepharose 4BCL/Protein A 210. Suitable filters are positioned in the columns as described in reference to FIG 4 The fluid leaving through outlet 211 has a reduced level of antibodies and complexes. The fluid is returned to the patient via catheter 212 and vein 217 When the plasma cell separator is in use the cellular elements of the blood are returned to the patient via line 213, catheter 214 and vein 215.

The columns can be connected to the patient in parallel, rather than consecutively, as illustrated in FIG. 6.

With either the manual or automatic operation of valving, the patient's blood or plasma can be transferred to column 301 and 302 either consecutively, with valve 303 open when valve 304 is closed and vice versa, or concurrently with valves 303 and 304 both open at the same time.

The method of Halbert, U.S. Pat. No. 4,637,880 may be used to regenerate one of two extracorporeal devices while the other device continues to be used, without removing either device from the mammal being treated, using any of the devices of the present invention.

In the utilization of the devices and methods of the invention, with or without the optional on-line regeneration step, heparin or another suitable anticoagulant may be administered intravenously or into the device as required, as is well known to those skilled in the art of extracorporeal treatment. See for example, Bensinger, U.S. Pat. No. 4,614,513.

Particularly when no regeneration of adsorbents is utilized, other columns can replace columns described in the current invention. For example, the column of Kuroda et al., U.S. Pat. No. 4,627,915 can be used to adsorb IgG and immune complexes, and the column of Ambrus et al., U.S. Pat. No. 4,612,122 or can be used to remove heavy metals.

The present invention also includes devices for carrying out the method of administering a drug bound (covalently or by other chemical binding) to an antibody such as an antibody specific to a tumor or to a tissue-specific antigen. Administration of the drug-antibody moiety is followed by a step of extracorporeally adsorbing the drug-hapten moiety by an antibody specific to the drug. The antibody in the extracorporeal device will thus adsorb both the drug-antibody moiety and the free drug in the circulation of the patient. The extracorporeal adsorption is preferably begun sufficiently long after the drug-antibody moiety is administered to permit the drug to reach a target in the mammal, although in some cases concurrent initiation of administration and adsorption is preferred. Generally, the time delay will typically be on the order of several minutes to forty-eight hours. An example of the drug is Adriamycin bound to a targeting antibody; the antibody in the extracorporeal specific affinity device will then be an antibody to Adriamycin. An example of the tissue specific antigens is thyroid gland specific antigen, an example of a tumor-specific antigen is human alpha-fetoprotein. Both the targeting antibody, to which the drug is initially bound, and the adsorbing antibody in the extracorporeal device may be an antibody fragment produced for example by synthesis or by enzymatic digestion treatment of a complete antibody. The adsorbent antibody is preferably linked to a matrix by a spacer arm of three to thirty carbon atoms, the targeting antibody is likewise preferably attached to the drug by a spacer. The adsorbing antibody may be made as a mirror image antibody, which binds to a site on the drug different from the site to which the targeting antibody is bound, by the method set out in my U.S. Pat. No. 4,375,414 Use of antibody to the drug in the extracorporeal device provides greater reduction in circulating drug (both bound and free), than does the antigen to which the targeting antibody is specific as used by Norrgren et al., supra

Numerous other variations in the devices and methods of the present invention, within the scope of the appended claims, will occur to those skilled in the art in light of the foregoing disclosure.

I claim:

1 An extracorporeal device for treating a disease state having at least two chemical species associated with it, said device including means for drawing from a mammal a fluid, means in said device for exposing at least a portion of the fluid to at least a first binding means and a second affinity binding means for chemically binding at least one of said chemical species in said fluid to the first binding means and for binding at least a second of said chemical species in said fluid to a second binding means, the first chemical species being a metal ion and the first binding means being a chelant, said second affinity binding means binding specifically to a species selected from the group consisting of an anticancer drug and an anticancer drug bound to a targeting antibody, and means for returning to the mammal at least a fraction of said fluid.

2. The device of claim 1 wherein said second binding means binds to a moiety consisting of an anticancer drug and a targeting antibody chemically bound to said anticancer drug directly or through a spacer, said targeting antibody being an intact antibody or a fragment of an antibody.

3 The device of claim 2 in which said specific affinity binding means is an antibody specific to said drug in the drug-targeting antibody moiety or a fragment of such antibody

4. The device of claim 2 wherein said specific affinity binding means is an antibody specific to a tumor or tissue targeting antibody of the drug targeting antibody moiety, or a fragment of such antibody.

5. The device of claim 2 wherein said specific affinity binding means is tumor antigen, or tissue antigen, or fetal antigen to which the targeting antibody is directed.

6 The device of claim 5 wherein said specific affinity binding means is human-alpha-fetoprotein.

7. The device of claim 2 wherein said drug in the anticancer drug targeting antibody moiety is adriamycin.

8. The device of claim 2 wherein said drug is radioactive.

9. The device of claim 1 wherein said drug is adriamycin.

10. The device of claim 1 wherein said drug is radioactive.

11. An extracorporeal device, including means for drawing fluid from a mammal, means in said device for exposing at least a portion of the fluid to at least a first and a second specific affinity binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to the second binding means, and means for returning to the mammal at least a fraction of said fluid, at least said first binding means being selected from the group consisting of antibodies to cholesterol, antibodies to oxidized LDL, oxidized LDL, malondialdehyde LDL, and chelants.

12. The device of claim 11 wherein the first binding means comprises fragments of antibodies to oxidized LDL.

13. The device of claim 12 wherein the first binding means is selected from the group consisting of synthetic fragments of antibodies and enzymatic digestion fragments of antibodies.

14. The device of claim 11 including a semipermeable membrane for preventing the specific affinity adsorbents from entering the body of the mammal.

15. The device of claim 11 wherein said fluid is blood.

16. The device of claim 11 including means for attaching the device into the blood circulatory system of the mammal.

17. The device of claim 11 wherein said fluid is plasma.

18. The device of claim 11 including means for exposing the fluid simultaneously to the first specific affinity binding means and the second specific affinity binding means.

19. The device of claim 11 wherein the second specific affinity binding means is a chelant which binds metal ions.

20. The device of claim 11 wherein the second specific affinity binding means binds a chemical species selected from the group consisting of autoantibodies and immune complexes.

21. The device of claim 11 wherein the second said affinity binding means is a non-immunological chemical affinity adsorbent.

22. The device of claim 11 wherein the first binding means comprises a chelant and the second specific affinity binding means is an antibody to an anticancer drug or a fragment of such antibody.

23. The device of claim 22 wherein said drug is adriamycin.

24. The device of claim 22 wherein said drug is radioactive.

25. The device of claim 11 wherein the first binding means comprises a chelant and the second specific affinity binding means binds to a moiety consisting of an anticancer drug and a targeting antibody chemically bound to said anticancer drug directly or through a spacer, said targeting antibody being an intact antibody or a fragment of an antibody.

26. The device of claim 25 in which the second said specific affinity binding means is an antibody specific to said drug in the drug-targeting antibody moiety or a fragment of such antibody.

27. The device of claim 25 wherein the second specific affinity binding means is an antibody specific to a tumor or tissue targeting antibody of the drug targeting antibody moiety, or a fragment of such antibody.

28. The device of claim 25 wherein the second said specific affinity binding means is tumor antigen, or tissue antigen, or fetal antigen to which the targeting antibody is directed.

29 The device of claim 28 wherein said specific affinity binding means is human-alpha-fetoprotein.

30 The device of claim 25 wherein said drug in the anticancer drug targeting antibody moiety is adriamycin

31. The device of claim 25 wherein said drug is radioactive.

32. An extracorporeal device, including means for drawing fluid from a mammal, means in said device for exposing at least a portion of the fluid to at least a first and a second specific affinity binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to the second binding means, and means for returning to the mammal at least a fraction of said fluid, at least said first binding means being selected from the group consisting of antibodies to cholesterol, antibodies to oxidized IDL, oxidized LDL, malondialdehyde LDL, and chelants, said device including means for exposing said fluid sequentially to the first specific affinity binding means and the second specific affinity binding means.

33 An extracorporeal device, including means for drawing fluid from a mammal, means in said device for exposing at least a portion of the fluid to at least a first and a second specific affinity binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to the second binding means, and means for returning to the mammal at least a fraction of said fluid, at least said first binding means being selected from the group consisting of antibodies to cholesterol, antibodies to oxidized LDL, oxidized LDL, malondialdehyde LDL, and chelants, at least the second specific affinity binding means binding immunologically.

34. The device of claim 22 wherein the second specific affinity binding means is selected from the group consisting of Protein A, Protein G, and C1q bound to anti-C1q antibody.

35. An extracorporeal device, including means for drawing fluid from a mammal, means in said device for exposing at least a portion of the fluid to at least a first and a second specific affinity binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to the second binding means, and means for returning to the mammal at least a fraction of said fluid, at least said first binding means being selected from the group consisting of antibodies to cholesterol, antibodies to oxidized LDL, oxidized LDL, malondialdehyde LDL, and chelants, the second specific affinity binding means binding a species, selected from the group consisting of cholesterol, triglycerides, LDL, oxidized LDL and antibodies to oxidized LDL.

36. An extracorporeal device, including means for drawing fluid from a mammal, means in said device for exposing at least a portion of the fluid to at least a first and a second specific affinity binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to the second binding means, and means for returning to the mammal at least a fraction of said fluid, at least said first binding means being selected from the group consisting of antibodies to cholesterol, antibodies to oxidized LDL, oxidized LDL, malondialdehyde LDL, and chelants, one of said first and second binding means being chemically bound to a matrix.

37. The device of claim 36 wherein the binding means is bound to the matrix through a spacer linked to the matrix by a spacer arm.

38. The device of claim 37 wherein the spacer arms comprises from three to thirty carbon atoms.

39 An extracorporeal device including means for drawing fluid from a mammal, means in said device for exposing at least a portion of the fluid to at least a first and a second specific affinity binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to a second binding means, means for separating at least said first chemical species from said first binding means to regenerate said device, and means for returning to the mammal at least a fraction of said fluid, at least the first specific affinity binding means being a chelant which binds metal ions and the second specific affinity binding means binding a species selected from the group consisting of cholesterol, triglycerides, LDL, oxidized LDL and antibodies to oxidized LDL.

40. A method for treating a disease state in a living mammal, the disease state being characterized by having at least a first chemical species and a second chemical species which are etiological to the disease state or to its symptoms, the method comprising drawing from the mammal a fluid containing both the chemical species into an extracorporeal device, exposing the fluid to at least a first adsorbent for chemically binding the first chemical species in the device and to a second adsorbent for chemically binding the second chemical species in the device, returning to the mammal at least a fraction of the fluid, and thereafter regenerating the device for further use by connecting a regenerating fluid to the device, and including a further step of administering to the living mammal intravenously at least one plasma component.

41. The method of claim 40 wherein the plasma component is gamma globulin.

42. A method of treating a disease state in a living mammal, comprising connecting into a fluid circulatory system of the mammal an extracorporeal device, exposing at least a portion of the fluid to at least a first and a second specific affinity binding means in the device for chemically binding at least one chemical species in the fluid to the first binding means and for binding at least a second chemical species in the fluid to the second binding means, and returning to the mammal at least a fraction of the fluid, at least the first binding means being selected from the group consisting of antibodies to cholesterol, antibodies to oxidized LDL, oxidized LDL, malondialdehyde LDL, and chelants.

43. The method of claim 42 wherein the first binding means comprises fragments of antibodies to oxidized LDL.

44. The method of claim 43 wherein the first binding means is selected from the group consisting of synthetic fragments of antibodies and enzymatic digestion fragments of antibodies

45. The method of claim 42 including a semipermeable membrane for preventing the specific affinity adsorbents from entering the body of the mammal.

46 The method of claim 42 wherein the fluid is blood, the method including attaching the device into the blood circulatory system of the mammal.

47. The method of claim 42 wherein the at least a portion of the fluid is plasma

48. The method of claim 42 including means for exposing the fluid simultaneously to the first specific affinity binding means and the second specific affinity binding means.

49. The method of claim 42 including means for exposing the fluid sequentially to the first specific affinity binding means and the second specific affinity binding means.

50 A method of treating atherosclerosis comprising a step of placing in an extracorporeal affinity adsorption device at least one affinity adsorbent which binds selectively with a chemical species which is etiological to atherosclerosis, wherein the chemical species is selected from the group consisting of oxidized low density lipoproteins and autoantibodies to oxidized low density lipoproteins, and a step of connecting the device in the blood circulatory system of a mammal to expose at least a portion of the blood of the mammal containing said chemical species to said affinity adsorbent.

51 A method of treating atherosclerosis comprising a step of placing in an extracorporeal affinity adsorption device at least one affinity adsorbent which binds selectively with a chemical species which is etiological to atherosclerosis, wherein the affinity adsorbent is selected from the group consisting of oxidized low density lipoproteins, malondialdehyde low density lipoproteins, antibodies to oxidized low density lipoproteins, antibodies to cholesterol, Protein A, and Protein G, and a step of connecting the device in the blood circulatory system of a mammal to expose at least a portion of the blood of the mammal containing the chemical species to the affinity adsorbent.

52 A method of treating atherosclerosis in a living mammal, comprising connecting into a fluid circulatory system of the mammal an extracorporeal device, exposing at least a portion of the fluid to at least a first and a second affinity binding means in the device for binding LDL in the fluid to the first binding means and for immunologically binding a second chemical species etiological to atherosclerosis in the fluid to the second binding means, and returning to the mammal at least a fraction of the fluid.

53. The method of claim 52 wherein the second species is selected from the group consisting of cholesterol, triglycerides, oxidized LDL, antibodies to oxidized LDL, and metal ion oxidants.

54 The method of claim 53 wherein the second species is selected from the group consisting of oxidized LDL and antibodies to oxidized LDL.

55. The method of claim 52 wherein the first adsorbent is a specific binding adsorbent which binds LDL selectively.

56. An extracorporeal device, including means for drawing fluid from a mammal, means in said device for exposing at least a portion of the fluid to at least a first and a second specific affinity binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to the second binding means, and means for returning to the mammal at least a fraction of said fluid, said first binding means being selected from the group consisting of antibodies to LDL, antibodies to cholesterol, antibodies to oxidized LDL, oxidized LDL, malondialdehyde LDL, and chelants, and said second species being selected from the group consisting of cholesterol, triglycerides, LDL, and oxidized LDL.

57 An extracorporeal device, including means for drawing fluid from a mammal, means in said device for exposing at least a portion of the fluid to at least a first and a second specific affinity binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to the second binding means, and means for returning to the mammal at least a fraction of said fluid, said first binding means being selected from the group consisting of antibodies to LDL, antibodies to cholesterol, antibodies to oxidized LDL, oxidized LDL, malondialdehyde LDL, and chelants, and said second specific affinity binding means binds immunologically.

58. An extracorporeal device including means for drawing fluid from a mammal; exposure means in said device for exposing at least a portion of the fluid to a binding means for chemically binding at least one chemical species in said fluid to the binding means, the binding means comprising an adsorbent bound to a matrix or a polymerized antibody, the exposure means comprising a first semipermeable membrane for preventing the adsorbent from entering the body of the mammal; regeneration means for regenerating said device, the regeneration means comprising a second semipermeable membrane, the second semipermeable membrane being nonpermeable to the matrix or polymerized antibody; and means for returning to the mammal at least a fraction of said fluid.

59 An extracorporeal device including means for drawing fluid from a mammal; exposure means in said device for exposing at least a portion of the fluid to at least a first and a second binding means for chemically binding at least one chemical species in said fluid to the first binding means and for binding at least a second chemical species in said fluid to a second binding means, at least one of the first and second binding means comprising either an adsorbent bound to a matrix or a polymerized antibody; regeneration means for regenerating said device, the regeneration means comprising a semipermeable membrane distinct from the matrix; and means for returning to the mammal at least a fraction of said fluid.

60 The device of claim 59 wherein the exposure means comprises a second semipermeable membrane for preventing the first and second binding means from entering the body of the mammal

61 The device of claim 59 wherein at least one of the binding means is a chelant which binds metal ions.

62 The device of claim 59 wherein at least one of the binding means binds a species selected from the group consisting of cholesterol, LDL, oxidized LDL and antibodies to oxidized LDL.

63. The device of claim 62 wherein the other binding means binds a chemical species selected from the group consisting of autoantibodies and immune complexes.

64 The device of claim 59 wherein at least one of the binding means binds immunologically

65. The device of claim 64 wherein at least one of the binding means is selected from the group consisting of Protein A, Protein G, and C1q bound to anti-C1q antibody

66 The device of claim 59 wherein at least the first said affinity binding means is a non-immunological chemical affinity adsorbent.

67. The device of claim 59 wherein one of said binding means is an antibody to an anticancer drug.

* * * * *

APPENDIX B - Provisional application of Meir Strahilevitz

METHOD FOR IMPROVED TARGETING OF LIGANDS WHEREIN THE LIGAND COMPRISES A TARGETING MOLECULE, TO A SITE IN AN ORGANISM, PARTICULARY A CANCER SITE, COMPRISING AFFINITY BINDING OF AT LEAST ONE OF THE GROUP COMPRISING OF TUMOR CIRCULATING ANTIGEN, ANTIBODY SPECIFIC TO TUMOR ANTIGEN, COMPLEX OF TUMOR CIRCULATING ANTIGEN AND ANTIBODY TO TUMOR ANTIGEN AND ANTIBODIES SPECIFIC TO AT LEAST ONE CONSTITUENT OF THE LIGAND

The field of the invention is targeting of ligands to a site in an organism, particularly a cancer site, by utilizing adsorbents with selective or specific affinity to chemical species , wherein the adsorption of at least one molecule is associated with improved targeting to the site in the body , such as a tumor site and increased concentration of the ligand in the targeted site , relative to its concentration in the rest of the body.

The adsorbents are primarily incorporated in an extracorporeal device , but the field of the invention is not limited to Extra Corporeal Affinity Adsorption or Extracorporeal Affinity Dialysis (Extracorpreal Affinity Dialysis is a method involving both dialysis (and/ or filtration) and adsorption as detailed in US patent 5,753,227 , see in particular ; column 5 line 40 to column 7 , line 44) , in that at least one of the adsorbents may be administered to the organism , such as by intravenous intraperitoneal, or other route and by binding of the "adsorbent" to the species , the species is cleared faster then it would otherwise clear from the body of the organism by increased elimination from the body, such as elimination through the kidney or liver or Reticulo Endothelial System (RES), or by increased metabolism and breakdown or neutralization of the chemical species.

The methods and devices that are describing the field of the invention and are relevant to elements of the invention that is the subject matter of the current application, include the following patents , patent applications and publications , all of which , including the references cited in said documents are incorporated herein by reference: US patents 4,375,414 and US 4,813,924 and any and all divisional applications or patents of said patents; US 6,039,946 and US 5,753,227 US 6,264,623 and published US patent application US 2001/0039392A1, US 2002/0019603 A1 and all their US divisional applications and issued patents and all equivalents and foreign counterparts of said patents and the divisional patent applications and divisional patents of said patents. PCT WO96/37516 and its foreign and US counterparts and all divisional applications thereof. For abbreviation all these patents and patent applications will be referred to at times as "my patents". Also incorporated herein by reference are the following patents and publications , including the references cited in them: Nillson et al: US patent US 6251,394 B1, V Pimm: Nucl. Med . Biol. Vol. 22, No 2, pp. 1020-1027, 1993, D. A. Goodwin et al: Antibody Immunoconjugates and Radiopharmaceuticals, Vol. 4, number 4, pp. 427-434, 1991, Van Kroonenburgh et al. , Nucl. Med. Commun. , Vol. 9, pp. 919-930, 1988, M Gurkavij et al., Cancer Research (Suppl.) Vol. 55, pp. 5874s - 5880s, December 1, 1995.
The limitations inherent in methods known to date, for the targeting of ligands , such as Treatment Ligands (TL) and Visualization Ligands (VL) [The definition of these terms is in accordance with PCT application WO 96/37516 its US and foreign counterpart and their divisional applications.] , to a site in an organism, particularly , but not exclusively tumor site. As known in the art of targeting

ligands to sites in the organism , such sites may be for example , an area of infarct in the heart , or a specific organ such as the Thyroid gland ,as examples. The tumor sites , in accordance with the current invention are both solid tumors that are tumors originating from cells outside the blood and the bone marrow (e.g., Leukemias and Lymphomas) as well as "soft tissue tumors" such as Leukemias and Lymphomas. Examples of solid tumors are well known and include , for example, colon carcinoma ovarian carcinoma pancreatic carcinoma as well as sarcomas. , as well as brain tumors, such as Asrocytoma and Glioblastoma .The treated organism which is a mammal, including , but not limited to a human, is conceptualized in accordance with the present invention to contain three compartments: The Tumor Compartment (TC) , The Blood Compartment (BC) and the rest of the organism : The Healthy Tissue and Healthy Organs Compartment (HC), including for example , the liver , kidney, spleen and lymph nodes. It should be realized that some or all of the organs may include tumor cell masses (such as metastases of tumor) . As used in the current invention the non tumor tissue and cells of the organ (kidney and liver for example.) will be referred to as HC and the metastatic cells (typically in the form of multiple cells) : are referred to as TC, even though they reside in the kidney or the liver , for example. It is clearly appreciated that the three compartments are in fluid communications, through , but not limited to the interstitial fluid (IF) , the capillaries of the blood circulatory system and the fluids of the Nervous System , Cerebro Spinal Fluid (CSF) and peritoneal fluid (PF) , for example. Further more , there is also fluid communications between the intracellular fluid (ICF) and the other compartments , including the above fluid compartments,for example the IF. It is also realized , that various chemical and cellular species , such as proteins , peptides, various antigenic and haptenic molecules, whether endogenous to the organism , or administered to the organism (such as TL and VL) various sub populations of lymphoid cells and macrophages, invading microorganisms such as viruses, bacteria and protozoa, are able to move from one compartment to the other , and may be at a steady state balance between the various fluid compartments and TC, BC and HC, meaning that removal of a molecular or cellular species from the BC, may for example change the rate of movement of the removed species from the other compartments to the BC.

In accordance with the present invention the tumor cells of the present invention contain Tumor Antigens (TA) and the Blood Compartment (BC) may contain Circulatory Tumor Antigens (CA) . It will be realized in accordance with the discussion above , that the CA, being a chemical species , usually will be able to migrate between the various compartments. A CA may be identical in chemical and antigenic structure to TA or it may have chemical and antigenic structure that is similar to but not identical to TA (for example the CA may have different affinity to specific monoclonal antibodies specific to TA then the affinity of same antibodies to TA). The body of the organism may produce antibodies to the TA and /or the CA. This antibodies are produced by the organism, as the result of the immune system in the organism mounting a humoral immune response directed at the TA and /or the CA . This antibodies are referred to as Native Antibodies (NAB) . NAB may act as "Enhancing Antibodies" (K. A. Hellstrom and I. Hellstrom: In: Encyclopedia of Immunology, I. M. Roitt and P. J. Delves Eds. Academic Press , 1998 p. 2440-2445) , such antibodies may bind to TA and mask the TA on the tumor cells, they may bind to lymphoid cell receptors and inhibit their ability to kill tumor cells, or they may participate in inducing suppressor lymphoid cells that inhibit tumor killing by Cytotoxic T cells (CTL) Examples of TA are given in WO 96/37516 and include Carcino Embryonic Antigen (CEA), Le(y), Alpha-Fetoprotein (AFT). Many other tumor antigens which include tumor specific antigens and developmental antigens (for example ovarian carcinoma CA-125 antigen) as well as monoclonal antibodies specific to these antigens are known in the art. (B. J Van

den Eynde and A. M. Scott: in Encyclopedia of Immunology , Supra pp. 2424-2430 .) In the presence of both CA and NAB , at least some of the CA and NAB bind to each other and produced CA-NAB complex . Such complexes may be in "antigen excess" (CA2NAB) , wherein the complex will generally have free antigenic sites available to bind additional antibody molecules, or it may be in "antibody excess" (CANAB2) , wherein the complex will generally have free antibody binding sites available to bind additional antigen molecules. CA-NAB , was found to play a role in the etiology and pathogenesis of cancer (F. A. Salinas and M. G Hanna , Jr. Eds. Contemporary Topics in Immunobiology, Vol. 15 :Immune complexes and Human Cancer, Plenum Press , 1985) and removal of CA-NAB by plasma exchange can be therapeutic (Immune complexes and Plasma Exchange in Cancer Patients, B. Serrou and C. Rosenfeld , Elsvier/ North-Holland Biomedical Press , 1981)

Factors that interfere with targeting TAB-bound VL or TL to a tumor site in an organism include:

(1) Presence of CA or CA2-NAB, that competes with the TA for binding of the TAB.

(2) TAB(bound to VL/TL) or CA-TAB (bound to VL/TL) complex binds to receptors such as Fc Receptors. If TAB is toxic, for example , is radioactive or is a toxin or toxic drug, this will lead to increased concentration of toxic VL/TL in healthy organs such as the liver , by binding of the TAB's Fc to Fc receptors on liver cells, when TAB contains Fc , such as when TAB is an intact antibody (it should be realized as mentioned earlier that TAB as defined in the current application may also be an antibody fragment , including synthetic fragment and fragment produced by genetic engineering. Complexes of such fragments with CA , even though they do not have Fc fragment, and do not bind to Fc receptors in the liver, they can still be cleared in the RES in the liver and elsewhere in Reticulo Endothelial System (RES) , by RES cells, such as macrophages.

(3) NAB compete with TAB for binding to TA and may inhibit TH1 Helper Cells' immune response to the cancer.

(4) CA-NAB complex concentrates in the RES, liver and kidney , for example , within the HC and toxic effects on normal organs is induced by this concentration.

(5) Particularly , following repeated administration of heterologus targeting antibody (eg: mouse monoclonal targeting antibody to human recipient) , the recipient may produce antibodies specific to the targeting antibodies . Such antibodies may be specific to the Fc part of the antibody (antiisotypic antibodies) or they may be directed to the Fv fragment (antiidiotypic antibodies) Such anti-targeting antibody antibodies (ATAA) can be produced by the organism, also when the TAB is a chimeric or humanized monoclonal antibody (M. V. Pimm Supra , Van Kroonenburgh et al., Supra.) ATAA may compete with TA for the binding of TAB.

It should be realized that depending on the particular cancer and the individual case, CA, CA-NAB complex , NAB, free ATAA TAB-ATAA complex (after the administration of TAB) may be present in various concentrations in the BC HC and TC. Optimally, particularly when these various concentrations are not known , it may be advantageous to remove or otherwise reduce more then one of the above species, and at least in some situations it may be desirable to remove as many of these species , as possible from the BC and/or HC and/or TC, prior to the administration of the TAB-VL or TAB-TL. Reduction of the amount of one or more of the above species in the BC and/or HC and/or TC, prior to the administration of the TAB-VL or TAB-TL , can preferably be achieved , in accordance with the present invention by Extracorporeal Adsorption , but may include , in some situations , reduction of one or more of the above species, by the administration to the subject of agents that would increase the breakdown and /or clearance from the body compartments of one or

more of the above species, that are known to increase such clearance from the organism (see , for example, D. A. Goodwin, Supra).

One approach that was used to reduce this interference is the administration of unlabeled targeting antibody prior to the administration of the labeled targeting antibody. This approach was associated with limited and various success (see for examples: M Helma et al. Cancer Immunol. Immunother. (1998) 47: 39-46, Beatty, B. G. et al. Cancer Res. 49, 1587-1594, 1989., Ahonen et al.: Acta Oncologica (1993), 32, 7/8 , pp. 723-7., Schrijvers A. G. H. et al.: J Cancer Research, (1993) 53, 4383-04390, September 15, 1993. Clearly significant improvement in specificity (e.g. : to target cell , target tissue or organ, relative to the rest of the body) of targeting is needed. Pre administration of unlabeled targeting antibody ,while binding to circulating antigen or to CA2NAB in the blood circulatory system (or in other biological fluid such as for example, cerebrospinal fluid (CSF) and peritoneal fluid) and thus reducing the amount of subsequently administered labeled antibody in the blood and /or in the liver , by reducing the amount of circulating antigen that can bind the labeled antibody, the pre administration of unlabeled antibody will also lead to the unlabeled antibody binding to the TUMOR ANTIGEN ON TUMOR CELLS and by this mechanism will REDUCE targeting to later administered labeled antibody to the tumor target .

Thus, generally Extracorporeal Adsorption (ECA) is preferred , in view of the fact that such a method is not associated with administration of additional species to the subject (that may have their own toxicity) and is not associated with increased concentration of TAB, TL or VL in healthy organs of the subject, associated with clearance, such as the liver and kidney. Generally the removal of one or more of the above species is completed 15 minutes to 48 hours prior to the administration of the TAB-VL or TAB-TL. It should be realized that the Targeting Ligand may be a species that is not an antibody species and its binding to the targeted site in the organism , may be based on none immunologic binding, as detailed and described in the incorporated references, in particular patens 6,039,946 and 5,753,227 and their counterparts and in Pimm , Supra, Gurkavich, Supra and Goodwin, Supra. Thus the targeting ligand may be a ligand that binds none immunologically to a receptor such as Epidermal Growth Factor Receptor, or it may be the peptide hormone Somatostatin , that binds to the Somatostatin Receptor , which is present in high concentration in many tumors. The targeting ligand can also be other targeting proteins or peptides (OTP) , or they can be none peptide targeting molecules such as a drug, for example Atenolol that binds to Beta-Adrenergic Receptor and Haloperidol, that binds the Dopamine 2 receptor

My US patents and patent applications Supra and in particular PCT applications WO 96/37516 provide for improved targeting over the previous art, by including a step of ECA of a species comprising circulating tumor antigen circulating tumor antigen-antibody complex and circulating (such as enhancing) antibodies specific to tumor antigen.

These methods will not be associated with competition of the administered unlabeled antibodies with binding of the later labeled antibodies to the tumor antigen at tumor sites . This methods will also not include the risks and undesirable effects of administering of unlabeled antibody to the organism, (infection , reaction to foreign protein Immune complex Disease). Optionally the methods of my patents Supra can also add a step of removing of the targeted treatment or diagnostic ligand by

extracorporeal affinity adorption at a predetermined time following the adsministration of the treatment ligand or visualization ligand to further improve targeting.
ECA removal of targeted ligands is disclosed in US patents 6,046,225 and 6,251,394 and PCT application WO 96/37516.

The removal of circulating immune complexes can be achieved in accordance with my patents supra by specific extracorporeal immunoadsorbents such as anticomplex antibodies , or by Protein A affinity adsorbents (Fresenius Immunosorba ®, Fresenius Prosorba ®, Fresenius C1q extracorporeal adsorbent Miro ®, Kaneka Selesorb ® as well as Asahi, as disclosed in US patent 4,627,915 as examples. Enhancing tumor antibodies can be removed for exmaple , using affinity adsorbents such as tumor antigen, Protein A Immunosorba ® , Prosorba ® , for example, Ciq , (Miro ® , for example) as well as use of the adsorbents as used in Selesorb ® and the above Asahi patent. Removal of circulating free tumor antigen (not complexed with antibody) can be achieved by the use of adsorbents such as specific antibody to tumor
antigen.

It will be realized that tumor targeting inhibitor factors (TIF) may exist in complexes containing more than two molecular species. Such complexes may be affinity labeled for adsorption or adsorbed in the ECA column, by an affinity label or affinity adsorbent specific to any of the components of the complex, or specific to epitopes that are specific to the complex. Examples of such complexes are: CA-TAB-ATAA and NAB-CA-TAB. For example, the complex CA-TAB-ATAA may be adsorbed by antibody to any of its three components, bound non-covalently (e.g., by ligand) to Protein A that is bound covalently to the matrix in the ECA column, or it may be adsorbed by Biotinylated antibody that is bound to Avidin in the Avidin ECA column. Similarly, the CA-TAB-ATAA can be affinity labeled for adsorption, by administering to the subject a Biotinylated antibody, specific to any of its three components, to enable its specific adsorption in the ECA Avidin column or affinity labeled by antibodies to any of the components of the complex (or antibodies to epitopes specific to the complex) and adsorbed by ECA on a Protein A column.

EXAMPLE 1
STEP ONE OF EXTRACORPOREAL REMOVAL OF CA-NAB, AND/OR NAB AND/OR ATAA PRIOR TO ADMINISTRATION OF TAB-VL OR TAB-TL

Protein A-Sepharose CL-4B obtained from Pharmacia LKB Biotechnology and swollen and washed in accordance with the instruction (Affinity Chromatography . Principles and Methods, Pharmacia Biotechnology Pub., 1991), The Protein A-Sepharose is packed in a column . Preferably the column used is the commercially available Immunosorba ® sold by Fresenius HemoCare Inc. Redmond, WA. The column requires the use of a plasma separator , as is well known and as recommended by the manufacturer: either a "centrifuge" type, such as Fresenius AS 104 cell separator, Cobe IBM 2997 or membrane type plasma separator, such as Kaneka Sulfox ® or Cobe TPE ®can be used to separate, on line, the patient's plasma from the cellular elements of blood. While the Immunosorba ® column is preferred in some applications , other Protein A Adsorption columns can be used instead. When regeneration is preferred a system including two Immunosorba ® columns with a Fresenius Automatic Regeneration unit , Citem 10 ® are used. When no regeneration of the adsorbent , column

is needed only a single Immunosorba ® column can be used. When it is preferable to use a disposable column , the column utilized for adsorption can be the Fresenius HemoCare Prosorba ® column in which the Protein A is covalently bound to a silica matrix. It should be realized that other Protein A columns can be used instead, using various biocompatible matrixes to which the Protein A is covalently bound. (Whenever Protein A is mentioned in this application the term includes , Peptide , fragments of protein A , including synthetic peptides, that are all known in the art to be used as equivalents of Protein A). The matrixes used include natural polymers, such as cellulose and dextran , various synthetic polymers and copolymers , such as polyacrylamide , polystyrene and polyvinyl polystyrene copolymer , and silica and silicones . One suitable matrix is heparinized silicone described in D. R. Bennett et al. US patent 3,453,194.

The configuration of the matrix is also not limited to a particular form , examples of suitable forms are beads (in particular, spherical in shape) , fibrous matrixes ,macroporous matrixes and membranes , including hollow fibers. One possible device is that of a typical multi-hollow fiber filtration, dialysis or diafiltration design. Such a configuration consists of a bundle of hollow fibers encased in a tubular housing. The adsorbent is included in the outer space of the hollow fiber , as disclosed in US patent 5.753,194, see in particular column 8, line 28 to line 68 and figs 2 and 3, the patent uses in the example a chelator as the adsorbent, but in the current invention the adsorbent may be Protein A, either in free form , bound to a matrix , particularly by covalent chemical binding .or alternatively the Protein A can be bound to the membrane, to its blood flow side to the side opposite to the blood flow or to both sides , rather then being incorporated in the outer space of the hollow fiber. The Protein A, in either free form , or matrix bound, can also be physically trapped in the membrane, (such as when the membrane is an unisotropic posysulfone membrane , for example the one produced by Amicon) When the Protein A is encapsulated it can be encapsulated in one of the microcapsules or macrocapsules as described in US patent 5,753,227 , in particular column 7 ,line 53-65, or the double encapsulation described by Markus et al. American Heart Journal, Vol. 110 No 1 ,part 1 , July 1985, pp. 30-39. In the practice of the invention adsorbents other then Protein A , or in some situations , in addition to Protein A , can be used , that can adsorb CA, CA-NAB, NAB or ATAA. These adsorbents include for example, TAB (that will bind CA , CA2NAB and ATAA.) Protein G, Ciq bound to anti-C1q antibody, covalently bound to matrix, C1g covalently bound to matrix (for example, Miro ® sold by Fresenius HemoCare) Dextran Sulfate (for example, Selsorb ® sold by Kaneka Corp.) and the adsorbent disclosed in Kuroda et al. US patent 4,627,915. Optionally , the Protein A Extracorporeal column 11 line 7. In the application of this method in accordance with the current invention an intact antibody , or an antibody fragment containing Fc fragment , and specific to a moleuclar species , desired to be removed , in accordance with the current invention, is bound to the protein A in the column by none covalent binding, following procedures known to those skilled in the art (see for example, E. Harlow and D Lane ; Antibodies , A Laboratory Mannual , Cold Spring Harbor Laboratory Pub. Pp. 411-522, 1988. and Affinity Chromatography, Principles and Methods, Pharmacia LKB Biotechnology Pub. # 18-1022-29,, pp. 47-52). In accordance with the present invention the antibodies bound to the protein A in the ECA column are one or more of the following specific antibodies (SA) : Antibody to TAB , antibody to CA (for example TAB) antibody to NAB, antibody to ATAA (e. g. anti idiotypic antibody), CA-NAB , NAB (anti idiotypic antibody) antibody to a tumor blocking factors and suppressor cells such as : TGF ß, p15E, TH2 T cell epitope.
In the EXAMPLE, the organism being treated is a human , the adsorption system used is the Fresenius system containing two Immunosorba ® columns and a Citem 10 ® regeneration unit. Each

column contains 62.5 ml of Protein A , which is covalently bound to a cross linked beaded Sepharose ® matrix. Plasma is first separated from blood cellular elements, using Fresenius AS 104 Cell Separator . The plasma flow rate in the Immunosorba ® column is approximately 20-35 ml/min (flow rates can vary , depending on the individual case and can be in a range from 5 to 50 ml/min, when Immunosorba ® column is used.

Depending on the individual case and the particular column used , persons with skill in the art can determine without undue experimentation the appropriate flow rate , in the individual case. Step One above can be completed preferably from 0.1 hours to 24 hours prior to the TAB-VL or TAB-TL administration. Most preferably it will be completed between 0.15 hours to 4 hours , prior to the TAB .administration. The length of step one is typically between 1 hour and ~17 hours and more typically between 2 hours and 4 hours, depending on the individual case including , for example , age , weight, presence of pathological fluids in body cavities , such as peritoneum, interstitial space, pericardium and pleural cavity, as examples. It should be understood that while in the EXAMPLE, plasma is being treated, other bodily fluids can be treated, such as peritoneal fluid, lymph, cerebro spinal fluid, with access to these biological fluids achieved by methods that are well known in the art. When fluid of the Blood Circulatory System is accessed and treated , the fluid may be blood rather then plasma (see for example US patent 5,753,227, including use of encapsulated adsorbent , when blood is directly treated) ..Following the adsorption STEP ONE, in the EXAMPLE, in STEP 2 , the TAB is administered intravenously. The TAB in the EXAMPLE is Hybrid of the two intact monoclonal antibodies Mab CHA-255 , specific to the hapten L-SCN-C6H4-CH2-EDTA and Mab ZCE-025 specific to CEA (C. Lollo et al. , Nuclear Medicine Communications, Vol. 15, pp. 483-491, 1994) The VL is 111 In-NBE-EDTA , which is bound none covalently to antibdy binding site of Mab CHA-255. The hybrid of Mab CHA-255 and Mab ZCE-025 is prepared by using the method described by Lollo et al. Supra, except that instead of the hybrid F(ab')2 , a hybrid of the intact antibodies (which is the preferred species used) is the hybrid utilized. Alternatively it is possible to use hybrid of one intact Mab with the Fab fragment of the second Mab thus including in the hybrid Fc piece of at least one antibody molecules , to enable binding to Protein A and Protein G , used as the adsorbent in the extracorporeal column . US patent 5,753,227, that is incorporated in this application by reference reads in column 10, line 51 to column 11 , line 8: "When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens. such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of rheumatoid arthritis. for example, the application of the analytical method of J. Goding et al.. J. Immunological Methods. vol. 20, 1978. pp. 241-53, to extracorporeal affinity adsorption in accordance with the present invention. is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al.. supra) is covalently bound to any of the matrixes described in the current invention. for example Sepharose 4B CL The antibody specific to the antigen. for example monoclonal anti-body specific to LDL is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part. and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the ECA column as described in the foregoing examples for the treatment of atherosclerosis, Clearly it is possible to use Protein G instead of Protein A in this system."

The adsorbent used in the CEA column may also be , Mab CHA-255 bound to Protein A or Protein G, through Fc of the Mab , EDTA that is covalently bound to the matrix , preferably through a spacer arm having a length of between 5-20 carbon atoms. Alternatively the EDTA can be conjugated to IgG that will bind to the Protein A through The IgG Fc. The EDTA will adsorb Free 111-In , released in the blood circulatory system. .

A modification of the method is to use liposome incorporation of VL in accordance with WO 96/37516. The incorporated ligand may be TAB-VL (For example, Mab ZCE-025-Mab CHA-255-NBE-EDTA-111 In) or it may be any other 111 In containing species, such as EDTA-111 In and target the liposome to the tumor , by binding to the wall of the liposome, covalently, or by ligand (non-covalently) Mab ZCE-025 , or its fragment that will target the liposome to the tumor.

EXAMPLE 2

Is identical to Example ONE above , except that the STEP 3 of ECA of TAB-bound 111 In NBE-EDTA- 111 In , or free 111 In is omitted.

EXAMPLE 3

Is identical to EXAMPLE 1 , except that prior to STEP 1 of ECA, in order to increase the affinity of the adsorbent Protein A , used in the EXAMPLE, monoclonal antibodies specific to the ATAA , (anti idiotypic antiboies to the FV binding site of ATAA), are administered to the treated subject . The production of monoclonal Anti Idiotypic antibodies to ATAA , are well known to those skilled in the art, as ATAA is a complete antigen . while use of monoclonal antibodies , preferably , chimeric or humanized antibodies are preferre, polyclonal antibodies can be used instead. Instead of administering anti ATAA antibodies , Cold TAB , e.g. TAB that is not bound to a VL or TL , can be administered , instead. In either case the production of ATAA-Anti-ATAA or ATAA-TAB complexes will increase their adsorption by Protein A, hence their effective removal by ECA. It is realized that should TAB be administered it will also inherently have affinity to and bind CA as well as CA2NAB.

EXAMPLE 4

Is identical to EXAMPLE 1 except that in order to enable the extracorporeal adsorption in STEP 1 of free circulating tumor antigen (CA) that are not bound to NAB and therefor can not be adsorbed by the Protein A used as adsorbent (as well as Protein G or C1Q, when these are used as adsorbents) as well as enable the more effective adsorption of ATAA, prior to step 1 of ECA , TAB (e. g. Antibodies specific to CA) is administered (intarvenously, intraperitoneally or by other route , depending on the individual case). TAB will also bind to ATAA (as the Antigen of ATAA) producing ATAA-TAB complex , thus enhancing the adsorption of the ATAA by the Protein A Protein G or C1q adsorbent in the ECA column. These TAB antibodies are preferably monoclonal antibodies , preferably chimeric or humanized antibodies, but can be polyclonal antibodies. The amount of antibody administered can be determined by those skilled in the art , depending on the individual case and will generally be between 0.1 Mg/Kg to 2 mg/Kg, Preferably 1 mg/Kg. The time interval between completion of this antibody administration and STEP 1 , is relatively short , in order to reduce to minimum the access of unlabeled TAB to the tumor, thus reducing the amount of unlabelled TAB bound to TA, and reducing the competition of unlabelled TAB , with the Labeled TAB . In view of

the fact that when unlabeled TAB is administered , such as by intravenous injection , it will have immediate access to CA in the BC , but a relatively delayed access to the tumor site , the time delay between completion of administration of unlabeled TAB and the initiation of STEP 1 ECA , will generally be between 5 minutes and 6 hours, preferably between 10 minutes and 2 hours. The time will depend on the individual case and can be determined by those skilled in the art without undue experimentation.

EXAMPLE 5

Is similar to EXAMPLES 1-4 , except that in order to enable adsorption of free NAB , by Protein A, Anti Idiotypic antibodies specific to NAB are administered prior to STEP 1 of ECA.

EXAMPLE 6

Is similar to EXAMPLES 1-5, except that in order to enable adsorption of tumor immunity molecular suppressors , (Such as TGFß and p15E) or tumor immunity cellular suppressors (such as TH2 suppressor cells), antibodies to the suppressor molecules and /or suppressor cells (e. g. In the example , antibodies to Th2 epitopes) is administered , preferably, prior to STEP 1 ECA.

EXAMPLES 7-12

Are similar to examples 1-6 except that the targeted ligand is a the TL Adriamycin , which is administered in accordance to EXAMPLE 2 of WO 96/37516, incorporated herein by reference.

EXAMPLES 13-19

Are similar to Examples 7-12 except that the Adriamycin is covalently bound to an intact Mab specific to the Tumor Antigen (TA) alpha-fetoprotein (AFP) and the method is in accordance with EXAMPLE 3 of WO 96/37516, incorporated herein by reference.

EXAMPLE 20 TO 26

The TAB is intact antibody ZCE-025 (see example 1) specific to CEA . It is directly Iodinated with 131 I , using the Chloramine-T (CT) method following the procedure of J. A. Carrasquillo et. al. Cancer Treatment Reports, Vol. 68, No 1, pp. 317-328, January 1984.

The TAB-131 I is administered in a dose containing 5 to 400 mCi radioactivity. The corresponding amount of Iodinated Mab is 0.65-52 mg. The other parameters of Tab administration are identical to those in EXAMPLES 1-6 In the above examples the 131 I TAB is used for TREATMENT.
The dose used for diagnostics is 5 to 15 mCi . The procedures for adsorption of CA, and/or CA-NAB and/or NAB and/or TAAA and/or TGFß and/or p 15E and/or other tumor suppression factors are identical to those described in EXAMPLES 1-6.

In all the EXAMPLES 1-26, wherein prior to STEP 1 ECA, the treated organism is administered a species (TAB, Antibody to TAA, Antibody to NAB Antibody to a Tumor Suppression Factor , such as TGFß and p 15 E , for example) aimed at production of a complex between the administered species and a Targeting-Inhibitor Species or a Tumor Immunosuppressor species. Rather then administering the species to the treated subject , the species (such as the TAB , antibody to TAA etc. Supra) , which antibodies comprise an Fc piece (preferably the antibodies are intact monoclonal antibodies) The species is bound to the Protein A adsorbent in the ECA column, rather then being administered to the organism, following the general method of using ECA with Protein A or Protein G bound to a specific antibody , in accordance with US patent 5,753,227 incorporated by reference

EXAMPLES 27 to 52

The procedure for the adsorption of CA and/or CA-NAB and/or NAB and/or TAAA is identical to examples 1-26. The TAB is identical to the TAB of EXAMPLES 20-26, except that the TAB is treated in accordance to the procedures disclosed in US patent 6,251,394, for the labeling of the TAB for post TAB administration adsorption., This patent is incorporated herein in its entirety , by reference. The labeling of TAB is preferably with Biotin.

The TAB may contain any of the Therapeutic or Diagnostic ligands described in the above patent (as well as those described in the current patent application in its entirety, including , but not limited to EXAMPLES 1-26 above and those disclosed in PCT WO 96/37516 .).

The subject being treated for therapeutic or diagnostic purposes is a human subject with a malignant melanoma tumor which is positive for the p97 surface glycoprotein antigen. The TAB is the monoclonal antibody 96.5 specific to p97. The TAB is labeled with 131 I and conjugated to Biotin in accordance with US patent 6,251,394. See column 8 , line 66 to column 9 , line 19:

> "The monoclonal antibody was the 96.5 (mouse IgG2a) specific for p97, a cell surface glycoprotein with the molecular weight of 97,000 present on 60-80% of human melanoma. The tumor model has been described in detail (Ingvar. C. et al. Nucl. Med 30. 1989, 1224). 2. Conjugation and Labeling of Monoclonal Antibodies. The monoclonal antibody 96.5 (330 ug) was labeled with s 37 MBq iodine-125 (125 I), using the Chloramine-T method. By elusion on a Sephadex G25 column (Pharmacia PD10) the fraction containing the labeled protein was collected and used for the conjugation. The labeling efficiency of the 125 I 96.5 was around 70%. The radiolabelled monoclonal antibody was conjugated with biotin by mixing 500 ug of antibody with 41 ug of N-Hydroxysuccinimido-biotin (NHS-biotin) in 0.1M NaHCO3, O.15M NaCl with 10% DMSO. The mixture was incubated for 1 h at room temperature, followed by overnight incubation at 4° C. The 125 McAb-biotin conjugate was separated from free biotin-reagent by gelfiltration on a Sephadex G25 column, equilibrated with PBS (10 mM Sodium phosphate, 0.15 M NaCl, pH 7.3). The conjugate was stored at 4° C until used.

The dose administered to the subject is the same as in examples 20-26 (5-400 mCi, 0.65-52 mg TAB). 4 to 48 hours, preferably 12 to 24 hours after injection of the TAB , the subject is treated by passing his blood through an Avidin adsorption column, preferably Mitradep ® column produced by Mitra Products, Inc.

The length of adsorption is in most cases between 1 hour to and 10 hours , most preferably , between 2 hours and 4 hours and depends on the individual case , including body weight of the subject and dose of TAB, and can be determined by a person skilled in the art with out undue experimentation. The volume of plasma treated is between 1 and 6 plasma volumes , most preferably between 2 and 4 plasma volumes. The flow rate is between 10-50 ml/min. A scintillation camera is used for imaging , when imaging is desired."

In the step of removal of CA, CA-NAB, ATAA, NAB , TGFß, P15E and other molecular and cellular Tumor Suppression Factors (TSF) , rather then using Protein A as adsorbent, Protein A bound to a specific antibody to the TSF (TSF molecules and/or cells TH2 suppressor cells epitopes , for example), in accordance with the general ECA method , wherein the adsorbent is Protein A bound to a specific antibody, as disclosed in US patent 5,753,227, incorporated by reference. Protein A bound to specific antibody, can be used instead of, or in addition to free Protein A adsorbent (e. g. Protein A unbound to specific antibodies.) Alternatively to the above option, in the step of removal of CA, CA-NAB, ATAA, NAB and other molecular and cellular TSF , a FIRST affinity adsorbent, other then Protein A or Protein G, can be used for binding to the FIRST affinity adsorbent a SECOND affinity adsorbent, specific to the TSF , in accordance with the general principle of US patent 5,753,227, in particular column 10, line 51 to column 11, line 80:

> "When affinity adsorption is used in accordance with the present invention for the adsorption of antigens or haptens. such as adsorption of LDL or oxidized-LDL in the treatment of atherosclerosis or adsorption of rheumatoid factor (autoantibodies to Human IgG) in the treatment of Rheumatoid arthritis. for example. the application of the analytical method of J. Goding et al.. J. Immunological Methods. Vol. 20, 1978. pp. 241-53. to extracorporeal affinity adsorption in accordance with the present invention. is a general method for the removal of antigens or haptens from the body. It should be clearly realized that any antigen or hapten can be removed from the body in accordance with this invention. In accordance with this method first Protein A or genetically engineered Protein A peptide (R. Lindmark et al.. supra) is covalently bound to any of the matrixes described in the current invention. for example Sepharose 4BCL. The antibody specific to the antigen for example monoclonal anti-body specific to LDL is added to the Sepharose-bound Protein A. It binds to Protein A through its Fc part, and its Fab antigen binding part is available to bind the antigen (LDL). The matrix bound Protein A-LDL-antibody is incorporated in the extracorporeal immunoadsosption (affinity adsorption) treatment column as described in the foregoing examples, for the treatment of Atherosclerosis. Clearly it is possible to use Protein G Instead of Protein A in this system."

For example the FIRST adsorbent in the ECA column is Avidin or Strepavidin to which is bound a SECOND specific adsorbent , comprising Biotinylated antibody to TSF. The use of Avidin-Biotin

combination, wherein the FIRST adsorbent is Avidin, was proposed by J Tennval et al. Cancer Suppl. Vol. 80 , number 12, pp.2411-2418,December15,1997, for the removal of ATAA , by adsorbing to the Avidin in the ECA column Biotinylated TAB. In accordance with the current invention, this approach can be utilized to remove any molecular or cellular TSF , by binding to the Avidin in the ECA column one or more Biotinylated antibodies specific to one or more molecular or cellular TSF. It should be realized that the general method of US patent 5,753,227, utilizing a SECOND specific adsorbent , bound none covalently (e.g. bound by ligand) to a FIRST adsorbent that is covalently bound to a matrix, is not limited to Protein A-specific antibody or to Avidin-Biotinylated specific antibody pairs. Various pairs of affinity ligands can be used as the FIRST adsorbent and the SECOND adsorbent. The First adsorbent , for example, is an antigen or an hapten covalently bound to a matrix. For example, Dinitrophenyl (DNP) bound to a matrix, preferably through a spacer, or a carrier molecule, such as albumin (e.g. albumin-DNP conjugate bound to the matrix) . The SECOND adsorbent can then be a hybrid antibody specific to both DNP and the TSF. The first adsorbent can be an antibody to DNP and the SECOND adsorbent an antibody to TSF covalently bound to DNP. The FIRST adsorbent can be an enzyme bound covalently to the matrix in the ECA and the SECOND specific adsorbent will then be an enzyme substrate or enzyme inhibitor conjugated to an antibody specific to TSF (or to another molecular or cellular species targeted for removal.) Similarly any of other known affinity pairs such as , for example, those listed in US patent 6,251,394 , column 7 , lines 54 to 67, can be used. Such Specific Adsorption methods using a FIRST and SECOND adsorbents can be used not only in ECA treatment, but also in adsorption-based purification or diagnostic methods, to remove any molecular or cellular species from a fluid, including but not limited to biological fluids.

EXAMPLE 53-56

is identical to EXAMPLES 5-8 except that the TL is the anti cancer drug Calicheamicin. The subject treated is a human being having a Acute Myeloid Leukemia positive for the CD33 antigen. The TAB is Recombinant Engineered Human Anti-CD33. The TAB is conjugated to the Calicheamicin in accordance to L M. Hinman et al. , Cancer Research , Vol 53, pp. 336-3342, July 15, 1993. 3336-3342, The dose of administered TAB-Calciheamicin conjugate is 6-9 mg protein/ m2 (E L Sievers et al., Blood Vol. 93 (11), June 1 1999).

EXAMPLES 56-60

Are identical to EXAMPLES 1-8, except that the Targeting molecule , in these examples is a none immunologic OTP, the hormone peptide Somatostatin. The treated subject is a human being having a cancer with high concentration of Somatostatin Receptors, as determined by biopsy . (C. Casini Raggi et al. Clin. Cancer Res. Vol. 8 (2), PP. 419-427, Feb 8, 2002.) Adraimycin is conjugated to the Somatostatin in accordance with A. Nagy et al. Proc. Natl. Acad. Sci USA, Vol 95, pp. 1794-1799, 1998, The dose of the conjugate is calculated to contain 30mg to 75mg Adriamycin/ m2 body surface. The post administration of Somatostatin-Adriamycin conjugate clearence from BC and HC is done with an ECA column containing antibody to Somatostatin bound to Protein A. Alternatively , the Somatostatin is conjugated also to Biotin in accordance with C M eppler et al. J. biol Chem, Vol 26 7(22), pp. 15603-12, August 5, 1992 and the conjugate administered to the subject is Biotin-Somatostatin-Adriamycin and the ECA is done with an Avidin column , for

examole , Mitra ® ECA column. The subject is treated after post conjugate administration so as to treat 1-5 plasma volumes , ECA is started 1-48 hours after Biotin-Somatostatin-Adriamycin is administered , preferably 2-24 hours . The length of post conjugate administration is 1-4 hours. The flow through the column is 20-50 ml/min.

EXAMPLES 61-64

Are identical to EXAMPLES 56-60, except that the subject being treated is administered a Yttrium-90 labeled Somatostatin analog prepared according to A Otte et al. The lancet, Vol 351 , pp. 416-417, February 7, 1998. The dose of the conjugate is 25 mCi to 200 mCi.

EXMPLE 65

A conjugate of Avidin with Mab ZCE-025 ,which is specific to CEA antigen is prepared according to H. P. Kalofonos et. al. : The Journal of Nuclear Medicine , Vol. 31, No 11, pp. 1791-1796, 1990. , except that the Mab ZCE-025 is substituted for Mab HMFG1. The subject being treated is having a tumor positive to the CEA antigen and has circulating CEA antigen in his blood. As determined by standard methods known in the art and available commercially . In STEP 1: The subject is administered intravenously (1-4 min) 0.1 mg protein/Kg to 2 mg protein/Kg of the Mab-Avidin conjugate. In STEP 2: The subject is treated with ECA with a Biotin Adsorbent Column (BAC) . The BAC ECA starts 0.5h to 48 hours , preferably 2-24 hours after the administration of the Mab-Avidin conjugate. 1-4 volumes of plasma are treated . The length of the ECA is 1-4 hours . The flow rate is 20 ml/min to 50 ml/min. For its use as the adsorbent in the ECA Biotin is covalently bound to a macromolecular carrier, such as albumin , using the method described in US patent 6,251,394, except that the albumin is substituted for the antibody. Albumin labeling with Biotin can also be done , following the method described in Harlow and Lane Supra, pp. 340-341, substituting the HAS for antibody. The Albumin-Biotin is covalently bound to Cyanogen Bromide Sepharose 4B beads available from Pharmacia. As an alternative to binding of Albumin-Biotin to Cyanogen Bromide activated Sepharose , The binding of the Biotnylated Albumin to Sepharose can be done by using Avidin-Biotin binding The albumin is biotinylated , so as to have sufficient Biotin moieties conjugated to the Albumin ,thus leaving sufficient number of Biotin molecules in the ECA column to be available to bind to Avidin in the Mab-Avidin conjugate. In STEP 3: The subject is given intravenously 0.5-10 mg protein of the conjugate Biotin -Human Serum Albumin(HAS)-131 I in 1-5 ml volume of 8.4% Sodium bicarbonate. Human Serum Albumin is directly Iodinated with 131 I according to E. Harlow and D. Lane : Antibodies A Laboratory Manual, Cold Spring harbor Laboratory pub. , pp.324-329, except that Human Serum Albumin is substituted for the antibody for the direct Iodination. The preferred Iodination is by the Chloramine T method (E Harlow and D Lane Supra, 328-329.)

In STEP 4: 0.5-48 hours , preferably 2 hours to 24 hours following the administration of the Biotin-HSA-131 I- conjugate , the subject is treated by ECA column ,that includes Avidin as the adsorbent , Preferably the Avidin CEA column is the Mitra ® ECA column. Length of ECA is 1-4 hours, Flow rate in the ECA is 20-50 ml/min.

The purpose of Steps 1 and 2 is to remove CA , CA2-NAB and TAA from the BC and HC and from the Interstitial Fluid of TC ,by specifically labeling CA CA2-NAB and TAA , with the TAB-Avidin conjugate and adsorbing the complexes TAB-Avidin-CA TAB-Avidin-CA2-NAB and TAB-Avidin-TAA by adsorption to the Biotin adsorbent in the Biotin ECA column. The Mab in the TAB-Avidin conjugate can be an intact antibody , antibody fragment , including synthetic fragment, and fragment produced by genetic engineering techniques. In addition to the removal of CA, CA2NAB and TAA, removal of any other molecular and/or cellular species can be accomplished by the labeling of the species with a conjugate of Avidin that is conjugated to an antibody to the species, such suppressor species include : NAB, CA-NAB2 , Transforming Growth Factor beta (TGFß) p15E factor, Interleukin 10 (IL-10), Prostglandin E2 (PGE2), Mucin, Suppressive E Receptor (SER) , Immunosuppressive acidic protein (IAP) and adhesion molecules. (K E Hellstrom and I Hellstrom, Encyclopedia of Immunology Supra and C Botti et al. Int. J Biol. Markers, Vol. 13 (2) , pp. 51-69, 1998.).

The use of a labeled affinity targeting molecule can be utilized by administration to the subject being treated to affinity label any molecular or cellular species , in particular in the BC but also in the HC and the Interstitial fluid component of the TC, provided that the species targeted for affinity labeling is in equilibrium between the BC , HC and TC compartment (unless the removal is desired only from the BC or from the other treated biological fluid compartment , such as peritoneal fluid CSF or lymphatic fluid, when this fluid is treated in the ECA device, when equilibrium with HC and TC is not required. It should be realized that usually , these species will be in a concentration equilibrium between the various compartments). With respect to the species that it is desired to remove in the treatment (or diagnosis) of cancer, , in addition to what was listed above: Cellular species include TH2 suppressor T cells that suppress the immune destruction of tumor cells. It will be realized that the disclosed "labeling -based adsorption", will have applications other then in the treatment of cancer, to remove any endogenous or exogenously administered or invading cellular or molecular species , such as auto antibodies in the treatment of autoimmune disease, sepsis associated factors, such as Tumor Necrosis Factor , Leukotrienes, Bradykinin and Interleukin 2, in the treatment of sepsis. Viruses and bacteria as well as protozoa in the treatment of infectious diseases. Toxins : e.g. Tetanus toxin , Butullinum toxin, for example. Other utilizations include : Affinity labeling for ECA of specific cellular and/or molecular species that are collected or harvested for therapeutic use , in the same subject , from which they are removed , or in another subject: For example , removal of specific T cell population , for in vitro treatment followed by readministration to the subject , in the treatment of cancer , e.g. In vitro stimulation of harvested T cells by treatment with Lymphokines ,in LAK or TIL cell treatment of cancer (See for example J. J. Sussman et al., Ann Surg oncol, Vol 1 (4), pp. 296-306, 1994 and S A Rosenberg et al. , Science , Vol. 233(4770), pp. 1318-21, Sept 1986.) The only requirement for such affinity adsorption harvesting is the availability or the ability to produce specific affinity labeling reagent that is specific to the target molecular or cellular species , and the ability to conjugate the targeting species to an affinity marker , such as Avidin (or Biotin) , for example. (It would be obvious that the method can be modified , by for example, the use of Biotin for Affinity labeling and use of Avidin as adsorbent in the ECA, or the use of affinity pairs other then Avidin Biotin, for example : anti hapten antibody - hapten , Enzyme -substrate and the likes.
One significant advantage of the proposed affinity labeling - affinity adsorption (ECA) , is that it can be possible to use one single ECA device (Avidin-ECA, Biotin-ECA, for example) to remove many

different species form the subject, by using different specific affinity labels targeted to the species to be removed and adsorbing them on the single device used for the ECA step of the method. Altern The different species can be removed at different times or at the same time .

For example: If the species is TAA it can be targeted by TAB-Avidin or by Anti-idiotypic antibody to TAA and remove by a Biotin-ECA. If , the species is Oxidized LDL it can be affinity labeled by administering a conjugate of Avidin-Antibody to oxidized LDL and removed by ECA with Biotin as the adsorbent. Pairs of affinity labels and their counterparts are known in the art and are disclosed , for example, in US patent 6,251,394 , column 6 line 7 to column 8 line 53. Many specific ligands in the Avidin-specific ligand conjugate are known and many are available from commercial sources. Generally , such specific ligands are known by , or can be produced by persons with ordinary skill in the art, following established methods and without undue experimentation. For example , if the specific targeting ligand is a Mab, many of the molecular and cellular species , that are affinity targeted for removal have known Mabs that are specific to them and many of them are available commercially. For example Mabs to CEA and other tumor antigens mentioned in the current application. Alternatively , such Mabs can be produced, without undue experimentation by those skilled in the art, by use of Hybridoma Mab production techniques. As an alternative to administering a affinity labels to the subject , the TSF affinity labels can be incorporated in the ECA column , utilizing an ECA method that incorporates as adsorbents a FIRST and SECOND adsorbents. For example the FIRST adsorbent may be Avidin or Protein A covalently bound to the matrix in the ECA column and the "SECOND" adsorbent may be a pleurality of biotinylated antibodies (when Avidin is the FIRST adsorbent, or untreated antibodies (when Protein A is the FIRST adsorbent) that are specific to at least two TSF species. Use of Double Stage Labeling of a tumor for Radiolabeling a cancer was reported in H. P. Kalofonos et al. , The Journal of Nuclear Medicine , Vol. 31, no 11, pp. 1791-1796, Nov. 1990. The authors labeled a lung cancer with a conjugate of a Mab specific to the cancer antigen, that was conjugated to Strepavidin and administered Biotin- 111 In to radio-label the tumor for diagnostic imaging. The authors did not disclose or hinted to the possible use of such labeling for the ECA of molecular and cellular species . Matrixes other then Sepharose can be used to produce matrix-biotin conjugate for use in ECA. For example G. Paganelli et. al. Disclose the production of biotinilated Nitrocellulose and biotinilated Polystyrene (G. Paganelli et al. Int J. Cancer Suool 2, pp. 121-125, 1988.).

Avidin ECA column can be used with whole blood rather then plasma, thus simplifying the adsorbtion as disclosed by J. Tennvall et al. Cancer Vol 80, No 12 (suppl.) pp. 2411-2418 Dec 15, 1997.

Following ECA on the Biotin adsorbent Column, the subject is administered a VL or TL conjugated to Biotin (it should be realized that the use of Avidin and Biotin in the example can be reversed; with the sequence of : Step 1: Administration of Biotin-TAB, Step 2: ECA on Avidin column Step 3: Administration of TL-Avidin or VL-Avidin conjugate. Step 4 ECA on a Biotin column. It would also be realized that affinity pairs other then Avidin biotin can be used instead of Avidin- Biotin pair such as , for example: Hapten -Anti hapten antibody, Enzyme-substrate, Enzyme-Enzyme inhibitor.) The VL-Biotin conjugate can be Biotin- 111 In and is prepared and administered to the subject according to Kalofonos et al. Supra: Biotin covalently conjugated to Diethylenetriaminepentaacetic

acid (DPTA) is obtained from Sigma chemical comp. St. Louis, Mo and chelated to 111 In as described in Kalafonos et al. Supra.

Example 66

In STEP 1 , the subject is a human with CEA positive cancer , as described in Example 65 is administered a conjugate of Avidin with Mab ZCE-025 specific to CEA.

In STEP 2, The subject is treated with ECA , incorporating biotin as the adsorbent. The purpose of this step is to remove CA, C2 -NAB and TAA.

In STEP 3, An anti-cancer drug , including ,but not limited to one or more of the anticancer drugs disclosed in PCT WO 96/37516 as well as the anticancer drugs disclosed in US patent 5,527,528, incorporated herein by reference is incorporated in a liposome prepared as described in US patent 5,527,528 , in particular , example 3 , for the preparation of Avidin coated liposome omiting the last step of incubating the Avidin coated liposomes with biotinilated antibodies. Optionally the Avidin is bound to the Biotin on the liposome wall through a spacer. Suitable spacers are disclosed, for example , in K Hashimoto et al. Biochim Biophys Acta, Vol. 856 (3), pp. 556-65, April 25, 1986. The anticancer drug incorporated into the liposome in the Example is Adriamycin.
Step 4 , The subject is treated by ECA with Biotin incorporated as the adsorbent , to remove liposomes from the BC, that did not reach or attached to the cancer.

Optionally Step 2 , and/or step 3 can be omitted .

Alternatively the liposomes are Biotinilated liposomes of US patent 5,527,528, optionally with the Biotin connected to the liposome wall with a spacer arm, the Adsorbent in the ECA of Step 2 is Aivdin and the adsorbent in the ECA in step 4 is Biotin.

Example 67

The subject is the same as in example 66. STEP 1 he is administered Mab ZCE-025, specific to CEA conjugated to Biotin following the method of US patent 5,527,528. In STEP 2 , The subject is treated with ECA incorporating Avidin as the adsorbent. In Step 3 , the subject is administered Ricin A conjugated to Avidin (L. K. Mahal et al. Science , Vol 276, pp. 1125-1128, 16 May, 1997.)

In STEP 4. The subject is treated with ECA , incorporating Biotin as the adsorbent.

In all of the Examples wherein the administered species is Avidin conjugate of TAB, or the administered species is Avidin conjugate of a none immunologic targeting molecule, in order to remove from the treated subject , when desired any of the species that would inhibit targeting and/or species that would in general suppress the immune destruction , or none immune mechanism destruction of the tumor , such targeting inhibitors and/or tumor destruction inhibitors can be removed from the BC , HC and TC by incorporating in the ECA adsorption column one or more adsorbents that have specific affinity to the targeting inhibitor or tumor destruction suppressive

molecular and cellular species. This can be accomplished by incorporating such specific adsorbents in a single column, or in different columns , connected in parallel or in series , as disclosed in US patent 5,753,227. When the ECA incorporates Avidin as the adsorbent , The specific adsorbent added to the ECA Avidin column is a Biotin conjugate of a specific affinity ligand (such as Biotin-Mab specific to CA, and /or ATT and/or any of the other suppressors as disclosed in Hellsrom and Hellstrom, And in botti et al. Supra.) When the adsorbent in the ECA column is Biotin, the Specific adsorbent added is Avidin-Mab specific to the CA and/or ATT, or other targeted inhibitors or tumor destruction cellular or molecular species as above.

EXAMPLE 68

In any of the Examples 1-67, in the step of removal of specific species from the blood circulatory system or from other biological fluid, such as, peritoneal and CSF, the specific Protein A -Specific Intact antibody adsorbent ligand,can be used for the removal of any substance from a biological fluid source, and for the application of the current invention, can be used for the adsorption- removal of any chemical species that may interfere with targeting, such as CA, CA-NAB, NAB, ATAA, or any molecular or cellular species that suppresses the Immune Destruction, or None Immune destruction of the tumor. The specific adsorbent is based on the method disclosed in US patent 5,753,227. Said patent is incorporated in the current application in its entirety. In accordance with the current example, intact antibodies, or antibody fragments, containing Fc,, wherein said antibodies or Fc containing fragments are specific to an epitope on the molecular or cellular species that is to be removed from the biological fluid and BC, HC and TC are added to a Protein A extracorporeal column, such as Immunosorba ® or Prosorba ® , or any other Extracorporeal column that contains Protein A, bound to matrix or encapsulated, as disclosed for example in US patent 5,753,227, supra. Such antibodies or Fc containing fragments, in the current example are selected from antibodies, or fragments specific to the molecular and cellular species, that inhibit targeting or suppress tumor destruction as disclosed in the current application and in Hellstrom and hellstrom and Botti et al., supra.

As disclosed in US patent 5,753,227 and also detailed in Harlow and Lane Supra ,pp. 519-523, the antibody or fragment will bind to the protein A in the ECA through the Fc of the antibody or fragment, thus producing a specific adsorbent ligand to specifically adsorb one or more of the Targeting-inhibiting molecular species, or molecular or cellular species that inhibit or suppress tumor destruction. This can be accomplished by incorporating such specific absorbents in a single ECA column, or in different columns, connected in parallel, or in series, as disclosed in US patent 5,753,227.